Assessing the soil health of farmers' conservation agriculture fields using the Biofunctool® index in South Africa

A Tempel

orcid.org 0000-0002-1848-9057

Dissertation accepted in fulfilment of the requirements for the degree *Master of Science in Environmental Sciences* at the North-West University.

Supervisor:	Dr L Muzangwa
Co-supervisor:	Prof GC du Preez

Graduation May 2024

Acknowledgements

To my dear friends and family, I extend my heartfelt gratitude for your unwavering love, support, and motivation throughout this journey. Your attentive ears and constant encouragement have been invaluable during my times of need.

I extend my thanks to Alexis Thoumazeau and the CIRAD Institute team for their invaluable assistance in training with the Biofunctool® and for granting me the chance to introduce this tool in South Africa.

Dr. João Marcelo Silva, your contribution to this project through statistical analyses is deeply appreciated.

A special acknowledgement goes out to all the farmers, especially Jaap Knot, for generously providing the study sites and graciously fielding my numerous inquiries about their management practices.

Dr. Gerhard du Preez, my co-supervisor, I am grateful for your enduring guidance and support.

Dr. Lindah Muzangwa, my supervisor, your unwavering support and encouragement during the challenging moments have meant the world to me. Thank you for believing in my potential, investing in my growth, and providing continuous guidance. Your support is immensely cherished.

Abstract

The conservation agricultural system offers distinct benefits compared to conventional agricultural management systems. Its capacity to lower input expenses, enhance soil's physical, chemical, and biological characteristics, and boost yields is known, with the potential to support sustainable intensification in numerous cases. However, due to the rising interest in managing agricultural fields for soil health and the profound reliance of humans on these ecosystem services, it is necessary to concentrate more on assessing the soil health condition of conservation agriculture fields to assure the applicability of field-level management strategies. To produce a robust interpretation of soil health, a mix of chemical, physical, and biological factors is commonly used. However, testing the major soil health indices is costly and time-consuming. As a result, the Biological Soil Functioning Assessment Tool, known as Biofunctool®, was created to assess total soil health by combining soil biota and physico-chemical parameters. The tool contains a collection of field indicators of three primary soil functions: soil nutrient cycling, carbon transformation, and structural maintenance, providing a holistic analysis of soil health status. Research has recognized that different soil organisms supported by soils play a significant role in the functioning of ecosystems. Therefore, researchers use soil enzyme analysis as a measure of soil health due to their close link to biological and physio-chemical features of the soil. The study took place in two contrasting agroecosystem regions, namely the North-West and Free-State provinces of South Africa, over a period of 4 seasons, namely, August 2021, March 2022, August 2022, and February 2023. The Biofunctool® parameters were only tested in August 2022 and February 2023.

The aim of this study was to assess the soil health of farmers' conservation agriculture systems in different agroecosystem regions, the Free-state province and North-West province of South Africa, using the Biofunctool® index and the activity of selected soil enzymes. The specific objectives were to (i) determine the effect of farming systems on ecosystem functions (i.e., soil carbon transformation, nutrient cycling, and structural maintenance) using the Biofunctool® soil health index (ii) determine the effect of farming systems on the activities of Fluorescein Diacetate (FDA) and β-glucosidase soil enzymes and (iii) to identify the relationship between parameters used in the calculation of Biofunctool® indices and selected soil enzymes.

The findings in our studies, for both the Biofunctool® and enzymes, highlighted a discernible gradient of soil disturbance, ranging from the conventional system to the conservation system

and finally to an uncultivated field characterised by minimal disturbance. The study highlighted the impact of various farming practices and seasons on soil functions. The soil quality index revealed that structural maintenance had the most significant influence on the overall outcomes. All the tested indicators improved under a CA system, with uncultivated fields having the highest results. By incorporating functional indicators, the Biofunctool® index effectively captures the intricate nature of the soil system and its reactions to various farming practices. The study of enzymes, SOC, and active carbon also revealed a clear continuum of soil disturbance, spanning from the conventional system to the conservation system and ultimately to an uncultivated field characterised by minimal disruption.

Keywords: Biofunctool®; conservation agriculture; soil enzymes; soil health; soil quality index

Table of Contents

Chapter 1: Introduction

1.1 Agriculture in South Africa

Meeting the food demand for an increasing global population with fewer external resources and less environmental impact is a significant challenge for the agriculture industry. Furthermore, efforts to maintain optimum crop production are hampered by several problems, such as drought, infertile soils, and high costs of fertilisers (Muhammad *et al.,* 2020, Thierfelder *et al.,* 2015). The difficulties are exacerbated by the need to adapt to the future climate conditions expected to be varied and extreme (Pittelkow *et al.,* 2015). Long-term consequences of climate change are anticipated to be detrimental due to increased temperatures and more frequent and prolonged droughts (Lee & Gambiza, 2022). For many people, especially in South Africa, agriculture is a source of income. Therefore, finding solutions for more sustainable crop management systems is necessary. These solutions must be practical at all levels of the community, farms and in the field, considering the intricate processes in which farmers operate and how their decisions can influence the environment (Thierfelder *et al.,* 2015).

The phrase "conventional agriculture" was referred to as everyday agriculture in the 1950s and 1960s and is still common. Regenerative, conservative, organic, and other types of agriculture are practised today in addition to conventional agriculture (Sumberg & Giller, 2022). Tillage and synthetic chemical inputs are two aspects of conventional agriculture. According to Tal (2018), conventional agriculture currently provides 98.9% of the world's food. However, conventional agriculture is a significant contributor to soil degradation on a global scale due to practices such as the removal of crop residues and soil tillage (Strauss *et al.,* 2021). Excessive and high-intensity ploughing has been practised in South Africa since the 1920s, which has led to increased physical, chemical, and biological soil degradation, including increased runoff, crusting, and compaction of the soil, decreased aggregate stability, restricted water infiltration, and decreased soil moisture availability (Thierfelder *et al.,* 2015). Monocropping, the yearly planting of the same crop in the same field, is another well-known practice of conventional agriculture (Thierfelder *et al.,* 2015). Recent studies have demonstrated that monocropping decreases soil microorganism populations, particularly in the topsoil, which results in loss of soil nutrients and increases reliance on synthetic chemicals to support crop growth (Mo *et al*., 2016; Panklang *et al*., 2022).

1.2 Conservation agriculture and soil health

Conservation agriculture (CA) is one of the more sustainable agriculture approaches known to alleviate the problems associated with conventional agriculture, including declining soil health (Sanaullah, 2020). Conservation agriculture refers to the simultaneous application of three main principles, namely reduced tillage, diversified crop rotation and maintenance of a permanent organic soil cover (FAO, 2022). According to Ranaivoson *et al.* (2017), a permanent organic soil cover physically protects the soil from extreme heat, raindrop impact, and wind forces. Additionally, residue retention provides food for the soil biota. Under CA, the roles of tillage and soil nutrient cycling are fulfilled by soil biota. For instance, earthworms are known to promote nutrient cycling, humus production, and the decomposition of organic materials (Mcinga *et al.,* 2020). They are also known as soil engineers because of their burrowing operations, which improve the passage of water and oxygen into the soil (Mcinga *et al.,* 2020).

A significant problem in South Africa is declining soil health due to detrimental farming methods and the alteration of weather patterns brought on by climate change (Thierfelder *et al*., 2015). However, CA is a potential approach to address this issue. Farmers might benefit from CA in various ways, including higher crop output and lower expenses (Ward *et al.,* 2017). Several farmers in South Africa have transitioned to CA. However, CA systems exhibit significant variability influenced by the context, management practices, and distinctive soil types at each site (Sumberg & Giller, 2022)

1.3 Soil health and soil ecosystem functioning

Research has linked the application of CA to improving soil health (Pheap *et al.,* 2019*;* Thierfelder *et al.,* 2018; Ward *et al.,* 2017). Soil health is defined as the "ability of soil to function as a living system, with the ecosystem and land use boundaries, to sustain plant and animal productivity, maintain or improve water and air quality, and promote plant and animal health (FAO, 2022). In general, healthy soils include a diverse community of soil organisms. These organisms can assist in the suppression of plant diseases as well as the management of insect and weed pests (Yang *et al.,* 2018). Soil organisms can also form a symbiotic relationship with plant roots, recycling vital plant nutrients. Additionally, they can enhance the structure of the soil, including its capacity to hold nutrients and water, which will ultimately enhance crop yields (Ranaivoson *et al.,* 2017). Therefore, it is critical to promote soil health and ecosystem functioning while ensuring the achievement of agricultural targets.

Soils can be considered a complex biomaterial with various functions that contribute to ecosystem services (Adhikari & Hartemink, 2016), including regulating, cultural, provisioning, and supporting services (Millennium ecosystem assessment (MEA), 2005). However, due to the rising interest in managing agricultural fields for soil health and the profound reliance of humans on these ecosystem services, it is necessary to concentrate more on assessing the soil health condition of CA fields to assure the applicability of field-level management strategies. To produce a robust interpretation of soil health, a mix of chemical, physical, and biological factors is commonly used (Obriot *et al*., 2016). Testing the major soil health indices is costly and time-consuming. As a result, the Biological Soil Functioning Assessment Tool, known as Biofunctool®, was created to assess total soil health by combining soil biota and physico-chemical parameters (Thoumazeau *et al*., 2019a). The tool contains a collection of field indicators of three primary soil functions: soil nutrient cycling, carbon transformation, and structural maintenance, providing a holistic analysis of soil health status (Thoumazeau *et al.,* 2019a).

Research has recognized that different soil organisms supported by soils play a significant role in the functioning of ecosystems (Sanuallah *et al.,* 2020). Therefore, researchers make use of soil enzyme analysis as a measure of soil health due to their close link to biological and physio-chemical features of the soil. The primary function of soil enzymes is to serve as catalysts for the recycling of organic matter, thereby offering insights into the activity of soil microorganisms (Rao *et al.,* 2017). Management of soil health, particularly in agricultural environments, can benefit from an understanding of the potential functions that various soil enzymes may play in sustaining soil health (Rao *et al.,* 2017). Soil enzymes such as the Fluorescein Diacetate (FDA) and β-glucosidase, are essential indicators of soil microbial activity and can help determine soil health (Karaca *et al*., 2010). They are important for maintaining the health of the soil ecosystem by aiding in nutrient cycling, degradation of organic pollutants, production of organic matter, stabilisation of soil structure, and life cycles of soil microorganisms (Das & Varma, 2010; Sanuallah *et al.,* 2020). The function of enzymes was added as a crucial indicator of soil health because soil biota plays a vital role in ecosystem functioning.

1.4 Problem statement

Conservation agriculture can solve agricultural challenges and has been embraced by several farmers in South Africa; however, there are differences in how it is applied from farm to farm (FAO, 2010). Furthermore, the ability of soil to perform soil functions depends on a variety of

factors, including the inherent soil qualities, the geophysical site circumstances, management history, and the adopted practices such as the integration of livestock (Creamer *et al.,* 2022). This suggests a need to evaluate the farmers' varying practices and their impact on soil health. Assessing soil health requires the selection of appropriate indicators that provide insights into soil ecosystem functioning. However, the indicators and techniques for evaluating soil health are constantly being reviewed and improved. Hence, testing the Biofunctool® introduced as a cost-effective, time-saving soil health tool can be quite effective. Furthermore, the use of enzyme analysis as a single indicator for assessing soil health presents an opportunity to streamline and expedite the evaluation process. However, there is a need to investigate the reliability and effectiveness of enzyme analysis in capturing the overall functioning of the soil ecosystem and its ability to reflect soil health accurately. The study took place in two separate agroecosystem regions, namely the North-West and Free-State provinces of South Africa.

1.5 Aims and objectives

The study aimed to assess the soil health of farmers' CA systems in different agroecosystem regions using the Biofunctool® index and the activity of selected soil enzymes.

The study objectives were to:

1. Assess the impact of farming systems on ecosystem functions, such as soil carbon transformation, nutrient cycling, and structural maintenance, across various seasons using the Biofunctool® soil health index.
2. Determine the effect of farming systems on the activities of Fluorescein Diacetate (FDA) and β-glucosidase soil enzymes across various seasons.
3. To identify the relationship between parameters used in the calculation of Biofunctool® indices and selected soil enzymes using Principal Component Analysis (PCA) for possible identification of soil enzymes as single-use indicators for soil health assessment .

1.6 Hypotheses

The specific hypotheses were as follows:

1. Farming systems managed by application of no-tillage, residue retention and crop rotation will improve the Biofunctool® parameters indicating better ecosystem functions, such as soil carbon transformation, nutrient cycling, and structural maintenance in different seasons .

2. Farming systems managed by the application of no-tillage, residue retention and crop rotation will present increased soil enzyme activity and there will be variations with season.
3. The application of PCA will reveal distinct and discernible relationship patterns amongst the measured soil enzymes and Biofunctool® parameters, and soil enzymes will be identified as single-use indicators for soil health assessment.

Chapter 2: Literature Review

2.1 Conservation agriculture

Conservation agriculture was introduced in the 1980s in African countries such as Zimbabwe (Thierfelder *et al.,* 2018), and the adoption of CA has increased globally since the 1990s (Mulimbi *et al.,* 2023). However, it was not until the early 2000s that CA was formally introduced in South Africa. According to a report by Kassam *et al.* (2018), CA was implemented globally in 2015/16 on more than 180 million hectares of cropland. Today, CA has been widely adopted in South Africa (Gura *et al.,* 2022; Lee & Gambiza, 2022; Thierfelder *et al.,* 2018) and has significantly increased, especially in dryland agricultural areas (Mulimbi *et al.,* 2023). Currently, the Western Cape is one of the leading provinces in South Africa with an adoption rate of 83% (Mulimbi *et al.,* 2023; Strauss *et al.,* 2021). According to recent research (Mulimbi *et al*., 2023), the amount of land used for producing crops under CA in South Africa increased by 366% between 2016 and 2019.

Conservation agriculture is built on the principles of crop rotation, crop residue management and zero tillage. This is interesting since CA contradicts conventional agricultural methods, such as the need for a clean seedbed free of crop residues (Giller *et al.,* 2009). In the face of climate change, CA aims to increase farmers' livelihoods (Mulimbi *et al.,* 2023). Crop residue retention is one of the methods used to achieve this, particularly in South Africa's arid environment, which creates a more humid microclimate for soil organisms (Thierfelder *et al.,* 2018). This technique can address issues including soil deterioration caused by agricultural methods that deplete soil organic matter and nutrients (Giller *et al.,* 2009). Conservation agriculture has been petitioned to take priority over conventional agriculture to improve soil health and ensure long-term agricultural productivity (Valbuena *et al.,* 2012).

2.1.1 *Principles of conservation agriculture*

2.1.1.1 *Permanent soil cover*

Crop residues are kept in the field and used as mulch. Mulching provides several benefits, including lowering maximum temperatures of soil, boosting aggregate stability, and promoting water infiltration and soil porosity (Sanaullah *et al.,* 2020; Thierfelder *et al.,* 2015). According to a study by Gura *et al.* (2022) on an Avalon soil form, leaving crop residues on the soil's surface enhances soil health. The decomposing residues replenished the soil with a sizable amount of plant-available nutrients. On a long-term basis, this helps to maintain soil fertility and plant nourishment. Crop residues provide a conducive environment for bacteria, fungi,

insects, and other macro- and microfauna and flora to flourish. As a result of these organisms' work, the mulch is broken down and added to the soil, enhancing its fertility over time (FAO, 2010; Fu *et al.,* 2021). Thoumazeau *et al.* (2019b) demonstrated that crop residues had a favourable impact on soil structure maintenance, contributing to the restoration of soil function following planting.

However, it is critical to understand the interaction of mulching and soil characteristics. Mulch use, for instance, can cause waterlogging in more humid conditions and on soils with poor drainage, lowering yield production (Giller *et al.,* 2009). Soils with a mulch layer tend to be moister and cooler, which, according to Sanaullah *et al.* (2020) favours the prevalence of small-seeded weeds and the breeding of pests and pathogens. Nevertheless, some research indicates that some crop residue may mitigate soil-borne diseases (Creamer *et al.,* 2022; Fu *et al.,* 2021; Yang *et al.,* 2019). A study by Yang *et al*. (2019) found that introducing garlic substrate to the soil produced a specific level of acceptable microbial variety that significantly aided in the fight against pathogens and greatly decreased the prevalence of diseases in the soil. In sandy soils, termites are more common in residue-covered fields. Termites are drawn to various mulches (such as leftover grass or maize) that contain crude protein and cellulose. Specialised termite species may damage some crops (Thierfelder *et al.,* 2015). Their habitats can be disturbed by tillage and residue clearance, reducing their energy sources (Thierfelder *et al.,* 2015).

Mulching has been demonstrated to be particularly successful in semi-arid areas (Giller *et al.,* 2009; Gura *et al.,* 2022). In southern Africa, the growing season's rainfall distribution patterns are characterised by mid-season dry spells (Thierfelder *et al.,* 2018). Therefore, CA has the potential to protect developing crops from the stress of periodic droughts by retaining surface crop residue which will increase infiltration rates and reduce the loss of moisture from the soil (Giller *et al.,* 2009; Thierfelder *et al.,* 2015).

2.1.1.2 *Diversified crop rotations*

Diversified crop rotation is defined as a set or multiple rotations of three or more crop species (Shah *et al.,* 2021) and is well known to improve soil health and reduce diseases and pest infestation (Sanaullah *et al.,* 2020:5). Monocropping of maize revealed a noticeably higher number of termites (Thierfelder *et al.,* 2016) and stimulates the spread of leaf diseases and permanent weeds (Sanaullah *et al.,* 2020). Therefore, alternating the crop species interrupts life cycles of pests by depriving them of their preferred host plants (Shah *et al.,* 202). A

synergistic relationship between different crops in rotation can lower input requirements, such as following a nitrogen-fixing crop with a high nitrogen-use crop (Sanderson *et al.,* 2013). Studies done by Thierfelder *et al.* (2015) emphasised the value of crop diversification and the inclusion of legumes in a crop rotation. Significant yield gains are reported in a maize crop planted after legumes and had nearly twice the yield as a continuous maize crop (Sanaullah *et al.,* 2020; Thierfelder *et al.,* 2015).

The value of employing soybean rotations was recently established by Gura *et al*. (2022) in their study. Soybean rotations enhanced the soil's nutrient characteristics because the residues were quickly converted to organic matter due to their low C:N ratios compared to maize and wheat rotations. According to research conducted in the Eastern Cape by Mtyobile *et al.* (2019), ammonium (NH_4^+) levels were found to be lower in continuous maize rotation than in a maize-fallow-soybean rotation. Grain legumes are also preferred in rotational crops due to adding nitrogen through biological fixation, and the additional grain yield is a source of food security and cash income. Based on a study done in Zambia, infiltration rates increased with a 2-year CA rotation of maize and cotton (Thierfelder *et al.,* 2016). It was also discovered that soils were more permeable and had less soil hardness when *Brassica napus* (canola) and *Lupinus perennis* (lupin) crops were used in rotations compared to a maize monoculture (Shah *et al.,* 2021).

Although there are many benefits of crop rotation, there are some restrictions. The soil type and the microbial community in the soil must be considered more so for some crops like *Cajanus Cajan* (Pigeon pea) and *Lablab purpureus* (Hyacinth bean), which promote termite activity (Thierfelder *et al.,* 2016). Additionally, planting crops in the areas of the landscape that will best support their growth can increase production efficiency, reduce sediment loss, and promote biodiversity conservation. It is essential to pay more attention to synergistic interactions within crop rotations and the complementarity of crops and landscapes to maximise diverse ecosystem functions within integrated systems (Sanderson *et al.,* 2013). Unfortunately, the slow adoption of rotational crops is typically caused by economic factors and dysfunctional input and output markets for seeds and produce (Shah *et al.,* 2021; Thierfelder *et al.,* 2016).

2.1.1.3 *Minimal soil disturbance*

It is well known that frequent tillage exposes the soil surfaces (Gura *et al.,* 2022; Sithole *et al.,* 2019; Thapa *et al.,* 2023). The exposed soils are vulnerable to wind erosion and the

disintegration of soil aggregates caused by raindrops (Hobbs, 2007; Sanaullah *et al.,* 2020). Tillage also disturbs the pores that roots and microbial activity created, causing the organic content of the soil to oxidise when exposed to the air. The soil beneath the surface can also be compacted by the tractor wheel (Hobbs, 2007; Sanaullah *et al.,* 2020). With CA systems, crops are planted directly into no-tilled soil, a practice known as no-tillage or zero tillage. Therefore, preserving soil organic matter lost during conventional agriculture by applying little soil disturbance is feasible. Besides providing the crop with additional nutrients, no-tillage stabilises the soil's structure and lessens the likelihood of the soil undergoing compaction and crusting, leading to erosion. In comparison to a field that has been traditionally tilled, evaporation of moisture is reduced under CA (FAO, 2010). A four-year study comparing CA and conventionally ploughed land in Ethiopia showed that CA decreased soil erosion and runoff (Thierfelder *et al.,* 2015).

However, there are reports of adverse effects after the reduction in tillage, such as increased weed pressure (Thapa *et al.,* 2023). Unless herbicides are used to manage weeds, this may increase the amount of labour required to remove the weeds (Giller *et al.,* 2009). Tilling can offer some benefits, including aerating the soil's organic content, preventing compaction temporarily, and eliminating pests and diseases (Hobbs, 2007). Termites are found to be more prevalent in areas covered in residue. Their habitats can be disturbed by tillage and residue clearance, reducing their energy sources (Thierfelder *et al.,* 2015).

2.2 Livestock inclusion under conservation agriculture systems

Conservation agriculture is used by farmers worldwide, particularly in the United States, Brazil, New Zealand, Africa and Australia, and for many, livestock is a significant source of income (Guesmi *et al.,* 2019). Small-scale commercial farmers in Africa and Asia regularly combine crop and cattle production in an integrated agricultural system (Valbuena *et al.,* 2012); however, this can lead to several difficulties for CA farmers. One of the main components of CA is that the soil is covered with some form of residue. The use of livestock rotation can influence mulching since livestock will use it as a source of feed (Guesmi *et al.,* 2019:4061; Valbuena *et al.,* 2012). Therefore, the management of livestock integration must be appropriately managed (Dhehibi *et al.,* 2023; Guesmi *et al.,* 2019). This can be done by combining diversified crop rotations with controlled, managed grazing (Dhehibi *et al.,* 2023). If grazing is not managed well, integrating livestock can lead to soil compaction, limiting crop yield (Sanderson *et al.,* 2013). It is also noted that overstocking of cattle can pollute

groundwater due to nitrogen leaching and enriching surface water with phosphorus (Sanderson *et al.,* 2013).

It has been proposed by Sanderson *et al.* (2013) to combine livestock and crops to provide environmental services for the rising population. The various benefits and drawbacks of using this practice were proven by an integrated crop-livestock system in the USA (Sanderson *et al.,* 2013). Compared to the non-integrated system (where farming activities are practised individually), the integrated system had a higher rainwater infiltration, decreased soil erosion, and required less chemical and N fertilisers. When cattle are specifically incorporated into an annual cropping system, the manure and urine can increase the levels of organic Carbon and Nitrogen in the soil (Guesmi *et al.,* 2019; Sanderson *et al.,* 2013; Thelen *et al.,* 2010). Crops produce residues to feed cattle; livestock gives draft power and manure for crop production. Especially in nutrient-deficient areas, the wise application of livestock manure could significantly increase crop output (Thierfelder *et al.,* 2015). However, it is crucial to effectively control the conflict between crop and livestock in a CA system. When incorporating livestock into the CA system, the current advice is to leave 30% of the mulch on the soil (Guesmi *et al.,* 2019; Smit *et al.,* 2021*).*

2.3 The challenges faced with conservation agriculture in South Africa

2.3.1 *The lack of resources*

Lee & Gambiza (2022) detailed the numerous challenges linked to CA. The initial investment required for CA can be relatively expensive, encompassing expenses such as heightened labour costs (Thierfelder *et al.,* 2015), specialised machinery acquisition, and the utilisation of herbicides and fertilisers. Another common obstacle pertains to education (Muzangwa *et al.,* 2017), with higher literacy levels playing a crucial role in comprehending new information about CA and recognizing its advantages and significance. It was observed that a higher level of education significantly enhanced the adoption of all three CA principles. With higher levels of education, it was found that the adoption of all three principles of CA was significantly better. One of the most crucial characteristics of a good CA practice is the mulch of crop residue. Crop residues, however, are a constraint for many farmers, particularly in Africa (Giller *et al.,* 2009; Lee & Gambiza, 2022; Smit *et al.,* 2021). Crop residue or mulch has multi-purposes, including fodder, construction materials, and fuel, and as a result, it is frequently in short supply. However, to fully implement CA, mulch must be used for fertility augmentation, weed/pest control, and rodent population control (Giller *et a*l., 2009; Smit *et al.,* 2021).

2.3.2 *Pedo-climatic concerns*

A pedo-climate is described as a soil microclimate that incorporates the impacts of the soil's temperature, water content, and aeration. Africa has several different climatic zones and soil types (Nortje & Laker, 2021), as shown in Figure 2.1. About 40% of the land on Earth is made up of arid regions, supporting approximately 38% of the human population (Trivedi *et al.,* 2016). Due to the over-exploitation of agriculture, these ecosystems may also see severe declines in nutrient availability, making them even more sensitive to global environmental changes and desertification. On the other hand, tropical agrosystems, known for their high crop productivity, may be remarkably resilient to agricultural uses because of their quick turnover of organic matter and easy access to moisture and water (Trivedi *et al.,* 2016).

Implementing sustainable farming technologies such as CA depends on the dominating soils and prevalent climates. The predominant soils and climates of developed and developing countries are very different from one another, with the former generally having better soil resources and weather than the latter for crop production (Nortje & Laker, 2021). This is so because the majority of developed countries, like North America and Europe, are located at relatively high latitudes in a temperate climate zone (Bationo *et al.*, 2012). Soils are deep, inherently fertile and have good physical conditions. Contrarily, the majority of the soils in developing countries, including South Africa, are of poor quality, including shallow soil and infertile sandy soil, and are typically located in hot and dry mid-latitudes (Nortje & Laker, 2021:1; Thierfelder *et al.,* 2018; van Antwerpen *et al.,* 2021). The soil map by Bationo *et al*. (2012) indicates Africa's major soil types (Figure 2.1). Xerosols, Lithosols, Solonchaks, Solonetz, Yermosols, and miscellaneous land units dominate South Africa. The soils are shallow, lack well-defined layers, and are made up of partially weathered fragments (Bationo *et al.*, 2012). These soils indicate that South Africa generally has lower soil fertility than countries such as North America and Europe (Nortje & Laker, 2021) and can make CA adoption difficult in some areas of South Africa.

Figure 2.1: A map showing the distribution of major soils in Africa. Note that South Africa is dominated by Cambisols and Lithosols (©FAO-GIS unit, published in Bationo *et al.,* 2012).

The soils of South Africa frequently have a granitic parent material, accounting for their sandy texture (Thierfelder et al., 2018). Most soils are poor in organic carbon and soil fertility (Strauss *et al.,* 2021). Aeolian sandy soils are predominant in regions like the North-West and northern Free-State Provinces of South Africa and are vulnerable to soil compaction (van Antwerpen *et al.,* 2021). According to du Plessis (2019), soil compaction is also one of the main yield-limiting factors in the North-Western Province's sandy loam soils. Numerous soils in southern Africa are also vulnerable to erosion and other forms of degradation. Only one cropping season, lasting from November to April, is usually practised in South Africa; the other months of the year are typically dry with little, inconsistent rainfall (Thierfelder *et al.,* 2018). Climate variability and change have a significant impact on South Africa, and predictions indicate that

these effects will become much more pronounced over the coming decades (Nortje & Laker, 2021; Thierfelder *et al.,* 2018). In the western Free State Province, severe dust storms were reported; it was calculated that 3 tonnes of productive topsoil were lost for every tonne of maize due to a severe drought that was evident in the 1960s (Strauss *et al.,* 2021).

Van Antwerpen *et al.* (2021) concluded that it was impossible to expect the successful CA systems reported in the Americas and Europe in South Africa without site-specific modifications. As mentioned in several studies, CA systems are superior to conventional approaches (Mulimbi *et al.,* 2023; Pittarello *et al.,* 2022; van Thierfelder *et al.,* 2018; van Antwerpen *et al.,* 2021). However, insufficient research has been conducted to adequately understand the suitability of CA and its benefits to agriculture and society. It also demonstrates that a variety of biophysical and socioeconomic constraints, which vary and rely on the location and the farmer's conditions, limit the promotion and acceptance of CA (Thierfelder *et al.,* 2015). Furthermore, benefits from CA are not always instant and take even years to be fully realised. It takes time for the soil to become physically and biologically healthy. It is suggested that it will take three to seven years for all the benefits to materialise (Hobbs, 2007).

2.4 Soil ecosystem functioning and soil health

The adverse effects of conventional agriculture have led to the promotion of soil management strategies focusing on improving soil health. Kibblewhite *et al.* (2008) describe that healthy soil must support food and fibre production at a rate and quality that can meet human requirements, as well as the ongoing provision of extra ecosystem services essential for maintaining biodiversity and human wellbeing. Soil management significantly impacts the soil characteristics and interactions that support ecosystem services in the soil. Therefore, it is necessary to understand how soils contribute to human wellbeing beyond just food production by integrating soils into the ecosystem services framework and connecting them to their many functions (Adhikari & Hartemink, 2016). Since soils are considered a living ecosystem, it is crucial to conduct evaluations similar to those of animal or plant environments (Haney *et al.,* 2018). Nutrient cycling, primary production, water regulation and purification, habitat biodiversity, and carbon regulation are acknowledged as the most important functions of soil in an agricultural system (Creamer *et al.,* 2022).

2.4.1 Evaluation of soil ecosystem functions

The chemical and physical components of soil, as well as some biological markers, have all been used to define the health or quality of the soil (Haney *et al.*, 2018). Two approaches to

understanding soil health can be categorised as either reductionist or integrated approaches. The reductionist approach is based on estimating the state of the soil using several independent indicators of particular soil qualities, including chemical, physical and biological ones, as seen in Figure 2.2. This method has been extensively discussed and reviewed and shares many characteristics with conventional quality evaluations in other disciplines, such as materials science (Kibblewhite *et al.,* 2008). The integrated approach works under the premise that soil health is more complex than a simple accumulation of contributions from a collection of particular components. It acknowledges that certain traits might develop due to interactions between different processes and properties. In Figure 2.2, the red reflects an integrative approach that concentrates on the functions of the soil directly from the interactions of the soil properties. In contrast, the green represents a reductionist approach, only focussing on the soil properties and not on the functioning of the soil (Kibblewhite *et al.,* 2008; Thoumazeau *et al.,* 2019a).

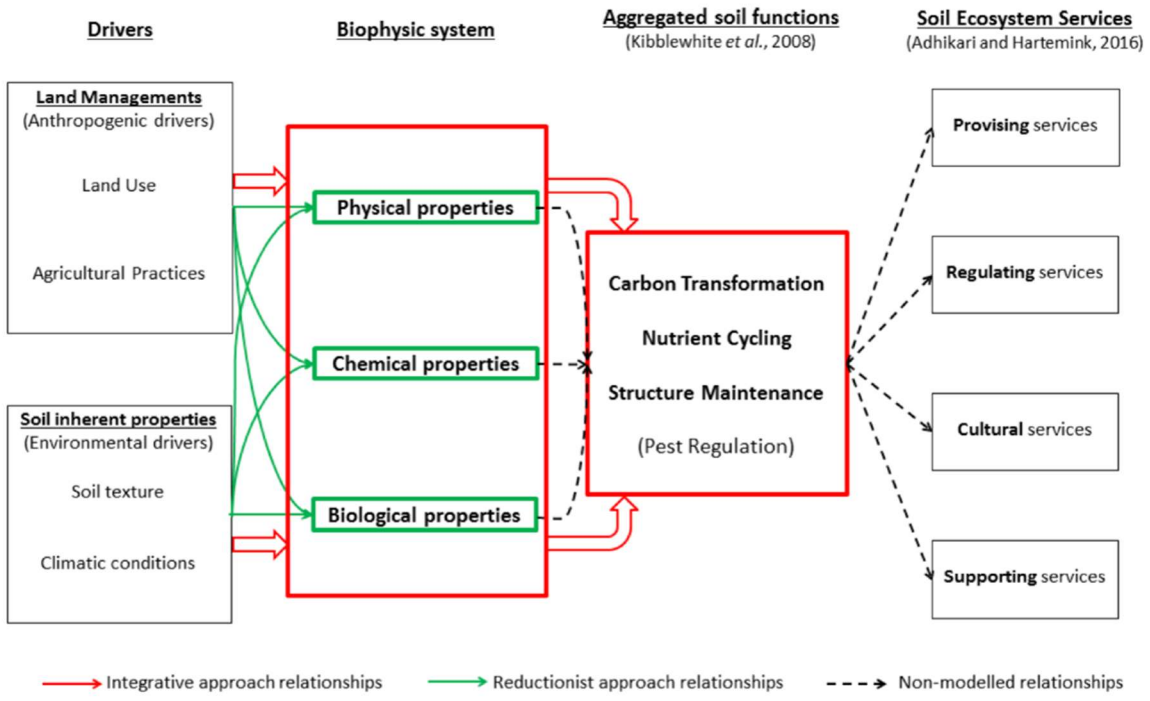


Figure 2.2: A framework outlining the steps of using an integrative or reductionist approach to evaluate soil health (Thoumazeau *et al.,* 2019a:101).

2.4.2 Biofunctool® index as a measure of soil ecosystem functions

The Biofunctool® was created using Kibblewhite's integrative viewpoint (Thoumazeau *et al.,* 2019a). The selection of indicators for the Biofunctool® was based on several criteria: first, they needed to capture the connections between biological populations and soil physico-

chemical properties to mirror an aspect of the overall soil functionality. Second, soil samples needed to remain undisturbed to depict the interactions within the soil system accurately. Lastly, the chosen measurements had to be efficient in terms of both time and cost, with no need for specialised skills (Thoumazeau *et al.,* 2019a). Therefore, the Biofunctool® contains a set of in-field indicators of the three primary soil functions: soil carbon transformation, nutrient cycling, and structural maintenance based on the integrative approach that includes the ecosystem functions.

The indicators included in the carbon transformation function (highlighted in brown, Figure 2.3) are permanganate oxidizable carbon (POXC), basal respiration (SituResp), and lamina. These indicators represent different stages of the soil carbon cycle's process. The available nitrogen (NminSoil) and the ion exchange membrane indicate the ability of the soil to cycle nutrients (highlighted in green, Figure 2.3). Nitrogen forms part of the Biofunctool® because it is the primary nutrient flux that microorganisms initiate and mediate through interactions with biotic and abiotic soil elements. The nitrogen and carbon transformation values inform the soil C:N ratio, a significant characteristic impacting soil function (Brady & Weil, 2014). The third set of indicators of the framework is the soil structure maintenance function (highlighted in blue, Figure 2.3), known as structural maintenance. The indicators include surface aggregate stability (AggSurf, 0-2cm), soil aggregate stability (AggSoil, 2-10cm), visual assessment of soil structure (VESS), and soil water infiltration (Beerkan). These indicators are connected to two major physical soil risks: soil compaction and erosion (Thoumazeau *et al.,* 2019a).

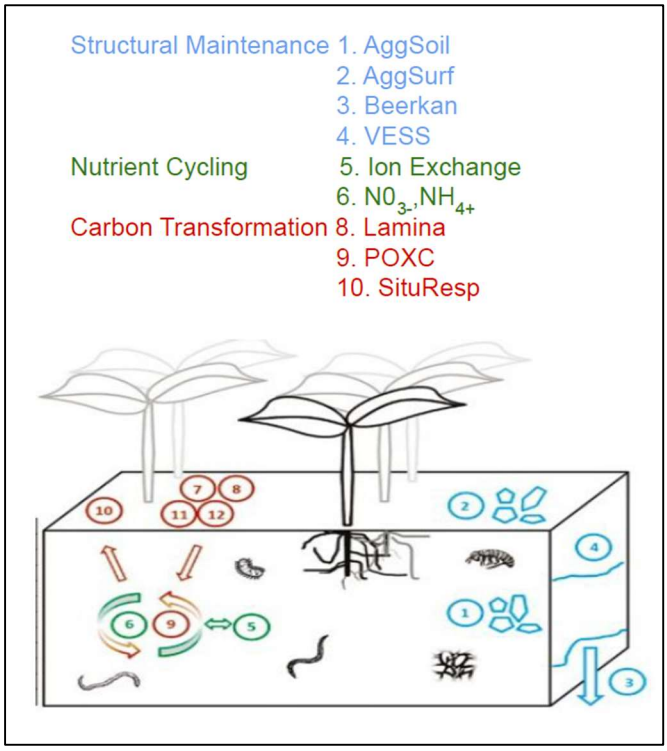


Figure 2.3: A schematic depiction of the Biofunctool® demonstrating their interactions in the soil (Brauman & Thoumazeau, 2020).

The Biofunctool® index, according to Pheap *et al.* (2019), is a useful method for assisting with decision-making to improve the management, effectiveness, and efficiency of cropping systems. The index is a useful tool for communication and is easy for a variety of stakeholders to grasp (including development practitioners, smallholder farmers, and policy-makers). The tool provides a comparative assessment of soil health within a specific context rather than an absolute evaluation of soil health (Thoumazeau *et al*., 2019a). This means that the Biofunctool® does not offer a definitive or absolute evaluation of soil health. Instead, it provides a relative assessment, comparing the soil's condition and characteristics within a specific context or against specific criteria.

2.4.3 Biofunctool® functions and related indicators

2.4.3.1 Soil structure maintenance

Soil structure maintenance refers to the techniques and approaches employed to preserve and improve soil's physical qualities. The soil structure is the way that distinct soil particles, such as sand, silt, and clay, are ordered and aggregated together. Plant development, water infiltration, availability of nutrients, and general soil fertility all depend on a healthy soil structure (Brady & Weil, 2014). Numerous studies have shown how the present farming methods are destroying the processes that contribute to soil fertility as well as soil structure

(Fiorini *et al.,* 2020). The indicators for this function are strongly correlated to soil erosion and compaction, which are two main soil concerns, particularly in South Africa, and are influenced by soil microbial communities to fit with the integrated approach (Thoumazeau *et al.,* 2019a).

2.4.3.1.1 *Aggregate stability*

Surface aggregate (AggSurf) and soil aggregates (AggSoil) determine aggregate stability by immersing the soil aggregates in a water volume. Aggregate stability is proportional to the soil structure's ability to withstand heavy rainfall events that could result in soil erosion or surface crusting (Thoumazeau *et al.,* 2019a). The aggregate stability method, designed by Herrick *et al*. (2001), meets the criteria for expressing management-induced changes in the soil structure and is rapid, repeatable, affordable, and simple to put together from readily available data. Soil aggregate stability is a significant measure of soil health due to its association with a range of ecosystem activities, features, and processes, including the amount and composition of SOM, soil biotic activity, erosion resistance, and infiltration capacity (Page *et al.,* 2020:4). The SOM fractions which are sensitive to management approaches have an impact on the stability of bigger macro-aggregates (Herrick *et al.,* 2001; Page *et al.,* 2020).

Although aggregate stability is beneficial and necessary in assessing soil health, it is rarely used. The methods for assessing aggregate stability can be expensive with certain sampling restrictions. Most approaches are laboratory-based, and facilities are not always available (Herrick *et al.,* 2001; Thoumazeau *et al.,* 2019). Sampling and transporting samples to laboratories can impose restrictions. The top 2 to 5 mm of soils contain the most critical aggregates for many ecological processes (Herrick *et al.,* 2001). Collecting and transporting these materials might be challenging without affecting the aggregate structure of the fragile surface samples.

It is crucial to understand that while this approach cannot wholly replace accurate laboratory-based assessments of soil aggregate stability, it can provide information in situations when traditional and time-consuming techniques are not feasible (Herrick *et al.,* 2001). Kulagowski *et al.* (2021) used the soil aggregate stability method and found that CA management significantly improved aggregate stability. Root exudates and residues that promote microbial activity improve the stability of the aggregates (Zuber *et al.,* 2017). Tillage destroys aggregates through slaking and pore-clogging, reducing the aggregate porosity and infiltration rates (Kulagowski *et al.,* 2021; Mattauer *et al.,* 2022). Aggregate stability can vary widely across many scales and soil textures, and this variability can serve as a reliable indication of soil ecological health (Sithole *et al.,* 2019).

2.4.3.1.2 *Visual assessment of soil structure (VESS)*

Visual assessment of soil structure (VESS) entails physically slicing up a portion of soil along the boundaries between aggregates. The soil slice is scored based on its size, shape, colour, roots, pores, and aggregates (Guimarães *et al.,* 2011) and is used to determine the soil horizons, the compaction states of the soil and the macro-organisms activity (Thoumazeau *et al.,* 2019a). Shepherd (2000) developed a simple, objective visual soil assessment method; however, it requires time and expertise. The arrangement of individual soil granules and the connections between aggregates determine the structure of the soil. Because of the reduction in intra-aggregate porosity, larger aggregates might promote inferior plant growth conditions (Guimarães *et al.,* 2011). Another method of determining the soil's structure is to examine the visible porosity inside aggregates using the naked eye (Guimarães *et al.,* 2011). To sum up, VESS is a tool that Biofunctool® uses to visually evaluate the strength of the aggregates, the size and porosity of aggregates, the existence of roots, and the colour of the soil (Kulagowski *et al.,* 2021).

2.4.3.1.3 *The Beerkan test (water infiltration)*

The Beerkan test measures soil water infiltration rate by introducing a set volume of water into the cylinder and then measuring the time it takes for the known water volume to infiltrate (Thoumazeau *et al.,* 2019a). The method aids in the knowledge of the hydrology of the vadose zone, which connects the surface water and groundwater components. The indicator is critical to understanding and defining the hydrological cycle. The test allows for figuring out the soil's hydraulic properties curves, which are the water retention and hydraulic conductivity curves (Lassabatère *et al.,* 2006:). Beerkan test is an in-field method to measure water infiltration rates and has proven to be more cost and time-effective than the soil cores, which often give poor approximations. Additionally, laboratory instruments are not well suited for revealing the hydraulic properties of the soil at a field scale (Lassabatère *et al.,* 2006:).

The structural maintenance of the Biofunctool® improved under CA techniques compared to traditional practice (Thoumazeau *et al.*, 2019b, Pheap *et al*., 2019, Kulagowski *et al.*, 2021). The positive infiltration rate results were directly related to aggregate stability (AggSoil and AggSurf) discovered under CA management (Kulagowski *et al.,* 2021:11). Since no-tillage and residue cover boost water infiltration, soil water infiltration will likely increase under CA systems (Page *et al.,* 2020:; Pheap *et al.,* 2019:). This is because tillage tends to reduce larger aggregates (Sithole *et al.,* 2019:). Water tends to move faster through a system with larger pores. Soil organic matter in the soil does not always form large aggregates. Under dry conditions, a large population of termites, millipedes, and beetles may positively impact soil

aggregation. Hence, under such conditions, high infiltration rates may be experienced under conventional systems, as demonstrated in a study by Sithole *et al*. (2019:).

2.4.3.2 Nutrient cycling

Nutrient cycling is part of the supporting services of ecosystem functioning (Adhikari & Hartemink, 2016; Creamer *et al.,* 2022). The nutrient cycle function can be described as the soil's ability to absorb and recycle nutrients from external sources while also aiding the soil community and enabling plants to obtain nutrients from soil minerals, water, and air (Creamer *et al.,* 2022). Nitrogen (N) is the principal nutrient flux that is initiated and controlled by microorganisms through interactions with the biotic and abiotic soil components and therefore provides an understanding of nutrient cycling. The primary focus is on the dynamics of readily accessible nitrogen forms, which directly result from interactions between soil components, making nutrient cycling crucial for integrative analyses. The nutrient cycling indicators and the carbon transformation indicate the soil C:N ratio (Graham *et al.,* 2014;Thoumazeau *et al.,* 2019).

2.4.3.2.1 ***Soil nitrogen availability***

Nitrogen mineralization involves transforming organic N into plant-available mineral forms (Creamer *et al.,* 2022:). It happens due to a biochemical conversion by microorganisms. Only heterotrophic microbes, which can function in aerobic and anaerobic environments, carry out the first phase (ammonification), where ammonium (NH_4^+) is produced. *Nitrosomonas* (which converts NH_4^+ into NO_{2-}) and *Nitrobacter* (converts NO_{2-} into NO_3^-) are two families of autotrophic aerobic bacteria that are primarily involved in the second phase (conversion of NH_4^+ into NO_3^-), also known as nitrification (Brady & Weil, 2014; Canali & Benedetti, 2003:). Ammonium (NH_4^+) and nitrate (NO_3^-) are the two most prevalent forms of nitrogen in the soil (Maynard, 1993). Since NO_3^- is the most prevalent inorganic forms of N, it is typically measured in well-aerated and cultivated soils. Usually, in waterlogged soils, where O_2 levels are low, NH_4^+ accumulates (Canali & Benedetti, 2003). Nitrogen mineralization is typically included in minimal data sets to assess a soil's ability to function within the confines of an ecosystem, notably to enhance biological productivity, preserve the quality of the environment, and protect the health of animals and plants (Brady & Weil, 2014; Canali & Benedetti, 2003).

Many recent studies have found that N is almost always higher under CA systems than in conventional systems (Kulagowski *et al.,* 2021; Pheap *et al.,* 2019; Smit *et al.,* 2021; Thoumazeau *et al.,* 2019b). This outcome could be explained by the increased decomposer

and microbial community activity under CA compared to CT (Pheap *et al.,* 2019). There could be a number of causes for the rise in NH_4^+ and NO_3^- in CA systems and can include the increase of N inputs via symbiotic N_2-fixing pathways or increasing the variety and quantity of organic inputs that may facilitate the activities of soil decomposers and microbial communities. The N status of the soil can also be impacted by no-tillage techniques that alter microbial activity related to the topsoil's N cycle (nitrifiers and denitrifiers) (Pheap *et al.,* 2019).

2.4.3.2.2 *Ion exchange membrane*

Ion exchange resins have been used to evaluate the quantity and rates of plant-available nutrient ions in soils since 1951 (Qian & Schoenau, 2002). Ion exchange resins act as a nutrient ion sink and are utilised to investigate the bioavailability of various nutrients in the soil (Le Cadre *et al.,* 2018; Qian & Schoenau, 2002). It can provide information on nutrient supply dynamics, including ion diffusion over longer distances, cation exchange, and slow nutrient release through dissolution or mineralization (Mettauer *et al.,* 2022; Thoumazeau *et al.,* 2019a). Under varying soil moisture and texture conditions, an ion exchange membrane can be used to show changes in nutrient transport to plant roots (Kulagowski *et al.,* 2021). Ion exchange resins in membrane or sheet form are simple to use. Burying them directly in the soil and examining the absorbed nitrogen serves as a highly effective indicator of N release potential among soils due to long-term management (Qian & Schoenau, 2002).

The study done by Kulagowski *et al.* (2021) discovered that a conventional field had two times the amount of nitrate adsorbed on the membrane of a CA field. The associated mixing up of residues with soil under conventional fields uncovers organic materials that were previously protected. This organic material acts as a substrate for microbial development, promoting mineralization and nitrification in an oxidised environment and explaining increased nitrate dynamics under conventional fields. However, the nitrogen dynamics brought on by tillage can lead to N losses through denitrification and nitrate leaching, particularly in areas of weak soil structure (Kulagowski *et al.,* 2021). In a study examining the impact of tree species on soil health, it was discovered that the levels of nitrates fixed on ion-exchange membranes were elevated in trees exhibiting greater litter inputs (Mattauer *et al.,* 2022).

2.4.3.3 Carbon transformation

Increasing the organic carbon content of agricultural soils is an effective way of improving soils (Basile-Doelsch *et al.,* 2020). Studies on soil carbon under CA systems are widely known due to the significant potential of soil sequestration to mitigate the effects of climate change (Pheap

et al., 2019:1; Sunaullah *et a*l., 2020). The indicators chosen for carbon transformation represent different stages in the soil carbon cycle and interact strongly with the biotic compartment of the soil; as a result, they can be taken into account in the integrative assessment (Kibblewhite *et al.,* 2008; Thoumazeau *et al*., 2019a).

One of the best ways of defining organic matter quality is to identify the different fragments or pools of organic carbon. These soil organic carbon (SOC) pools differ by how easily microbes can break them down. There are three main pools of the soil's total organic matter: the active, passive, and slow pools, as illustrated in Figure 2.4 (Brady & Weil, 2014). Soil organic matter (SOM) is about twice as large as the atmospheric C pool and is the largest carbon resource in a terrestrial ecosystem (Sunaullah *et al*., 2020).

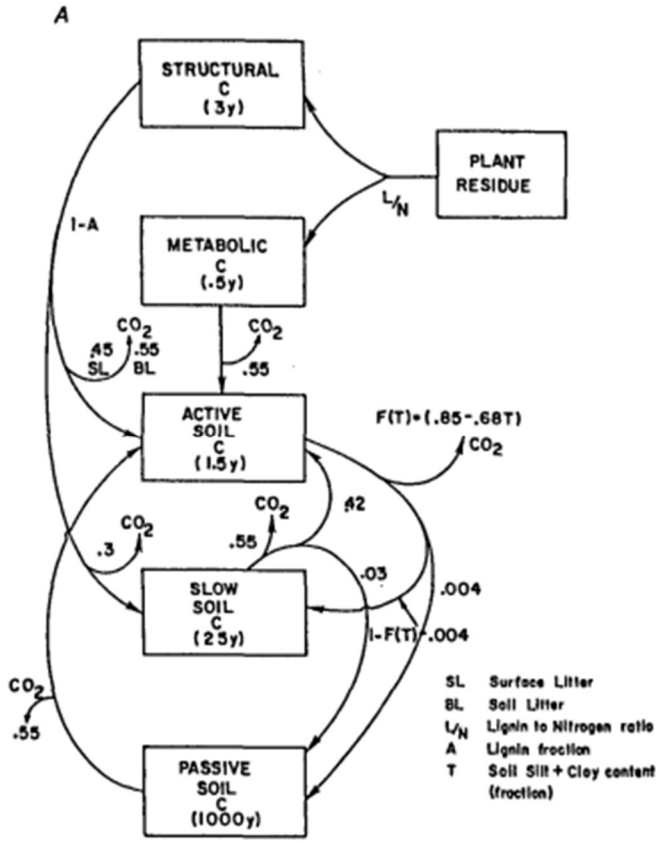


Figure 2.4: A conceptual model considering different SOM pools and their susceptibility to microbial metabolism (Paustian *et al.,* 1992).

Labile materials with half lifetimes of only a few days to a few years make up the active pool of SOM. The average C:N ratio of the organic materials in the active pool is relatively high. The active pool comprises polysaccharides, particulate organic materials, biological biomass, and other non-humic elements. The active pool is crucial since it supplies most of the rapidly mineralizable nitrogen and immediately available food for soil organisms. It is primarily

accountable for the favourable impacts on the structural stability of soil that result in improved water infiltration, protection against erosion, and ease of tillage (Brady & Weil, 2014). The microbial biomass, which is tightly linked to active pools of potential C and N mineralization, is the main force behind the breakdown of organic matter and the cycling of nutrients. Bacteria sequester C, N, and P in a ratio of roughly 100:10:1, and C is the primary driver of the soil nutrient-microbial recycling system (Haney *et al.,* 2018).

2.4.3.3.1 *Permanganate oxidizable carbon (POXC)*

The short-term turnover of the soil carbon pool can be indicated by permanganate oxidizable carbon (POXC) (Thoumazeau *et al.,* 2019a). Permanganate oxidizable carbon is a portion of the soil's organic matter that has undergone processing and is directly correlated with soil microbial activity (Culman *et al.,* 2012). Permanganate oxidizable carbon is linked to particulate organic carbon (POC) fractions that are smaller and heavier. Particulate organic carbon is an important C fraction that reflects crucial processes, including soil aggregation, nutrient availability, and soil carbon build-up. It is suggested that POXC represents a pool of active soil carbon (Figure 2.3) that has been processed or stabilised. Additionally, POXC is more responsive to management changes than other soil C components, suggesting that POXC may serve as a valuable marker for promptly identifying changes in SOM brought about by management (Hurisso *et al.,* 2016). Permanganate oxidizable carbon is a rapid, inexpensive test that can be altered for use in the field. Permanganate oxidizable carbon is a useful indicator for studies on soil health because it may be used to track management techniques that enhance soil C sequestration (Culman *et al.,* 2012).

A long-term trial in Cambodia showed how CA and conventional practices impacted POXC (Pheap *et al.,* 2019). The authors discovered that the POXC results under the CA systems were three times greater than those under the conventional system. This data is also supported by Hok *et al.* (2018), who observed a considerable increase in the POXC levels with crop rotations and cover crops after a short-term CA practice in Cambodia. The study concluded that the crop residues close to the soil surface provided a constant source of biomass-C (Hok *et al.,* 2018).

2.4.3.3.2 *Basal respiration (SituResp)*

Respiration, in a less technical sense, is the process of oxygen uptake while simultaneously releasing carbon dioxide (Pell *et al.,* 2003). It is connected to important functions of soil ecosystems, such as primary production, climate regulation, and nutrient cycling (Babur,

2019). Because there are multiple CO_2 sources, measuring soil respiration is highly complicated. Two of these sources are autotrophic respiration associated with root activity and heterotrophic respiration linked to soil microorganism activity. Basal soil respiration is the constant rate of CO_2 emissions associated with microbial decomposition of SOM in a soil absent from roots (Thoumazeau *et al.,* 2017). According to Pell *et al.* (2003), the continuous rate of respiration in the soil is known as basal respiration (BAS) and results from the breakdown of organic materials (predominantly native carbon).

The carbon supply's volume and quality are reflected in the BAS rate. Therefore, BAS can serve as a combined indicator of the soil biota's capacity to break down naturally occurring and anthropogenically introduced organic materials in a given environment. A crucial process for carbon transport into the atmosphere is soil respiration. The major elements that control soil respiration are the amount of water, oxygen, and carbon bioavailability (Pell *et al.,* 2003). Basal respiration has long been used as an indicator of how agricultural practices affect the biological activities of the soil (Thoumazeau *et al.,* 2017) and was found to be much higher in CA systems compared to conventional systems (Pheap *et al.,* 2019). Another study by Babur (2019) indicated that BAS was much higher in limestone soils. The increased BAS was linked to increased microbial activity, owing to the growth of the environment for microorganisms and the rise of labile soil carbon (Pheap *et al.,* 2019).

2.4.3.3.3 *Bait-Lamina*

The fauna in the soil is known to be involved in numerous soil processes, such as the building of soil structure and hence supporting a range of ecosystem functions. Erosion control, nutrient mineralization, and chemical detoxification are some of the ecosystem services supported (Gavin-Centol *et al.,* 2023). Fauna activity in cropping systems can be measured using bait-lamina (Vorobeichik & Bergman, 2020). The test involves filling holes with bait on a lamina strip (Figure 2.5) before inserting them in the topsoil for a given period and then counting the broken-down holes (Thoumazeau *et al.,* 2019a). The bait-lamina approach helps understand litter degradation and cellulose decomposition and demonstrates the effects of environmental pollutants. The method is a simple and low-cost way to assess soil biological activity (Kratz, 1998; Vorobeichik & Bergman, 2020). It explains how soil invertebrates and soil microbes interact with the nutrient cycle in terrestrial ecosystems. In an agricultural setting, bait-lamina can be used to assess how different cropping patterns affect soil arthropod feeding behaviours. The information helps researchers figure out how soil improvement, soil compaction, and fungicide treatment affect biological activity (Kratz, 1998). The bait-lamina

tests, according to van Gestel *et al.* (2003), are only suggestive of the activity of the soil fauna, particularly earthworms, and not of the soil microbiota.

A previous study by Kulagowski *et al.* (2021) observed considerably higher lamina-bait holes in a CA field than in a conventional field. In this study, they only identified the first four holes (0-2cm) since it was the only depth that allowed them to distinguish the activity of organisms and where the majority of feeding activity took place. In a CA system, this observation is explained by the impacts of no-tillage and the residues left behind by the cover crops on the soil surface and root systems. Another study done by Gavin-Centol *et al.* (2023) found that feeding activity was completely reduced in a conventional farming system compared to organic-managed fields.

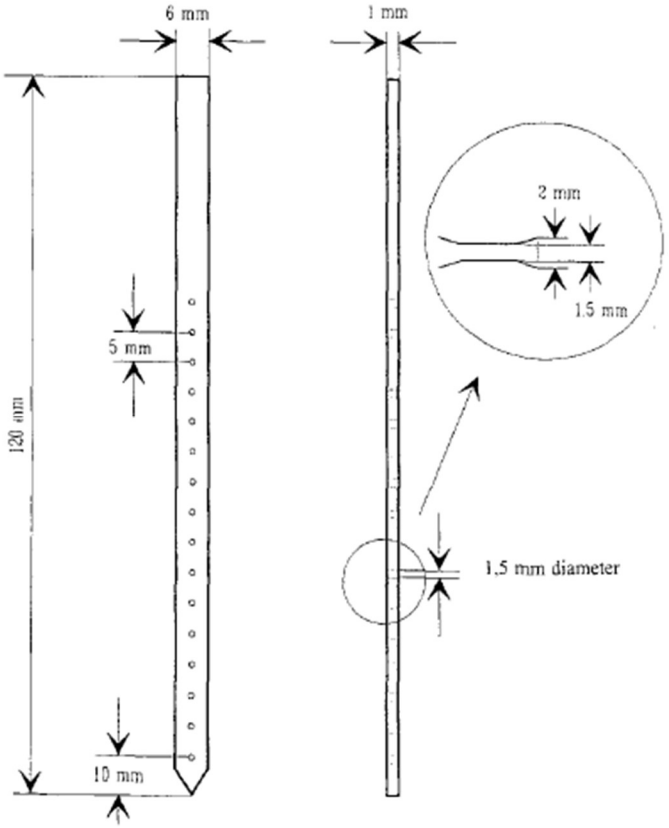


Figure 2.5: A small amount of substrate is placed in tiny holes drilled through PVC strips and then exposed to soil-based biogenic breakdown processes (Kratz, 1998:95).

2.5 Soil enzyme activity as a measure of soil health

Brady & Weil (2014) mentions that most work of the soil community is carried out by organisms whose "jaws" are chemical enzymes consuming organic materials left in the soil by their cohabitants. Soil enzymes play a crucial role in ecosystem functioning by providing various essential services. They are critical SOM decomposers and nutrient recyclers, and they have an impact on environmental quality, energy conversion, and crop production (Saccá *et al.,*

2017). Enzymes are found in a variety of places, including microbial cells, tiny animals' guts, and soil particles. Microbes release the majority of these enzymes into their immediate environment (Brady & Weil, 2014). Enzyme activity is critical since all biochemical processes in soil rely on or are influenced by the presence of enzymes (Riah *et al.,* 2014). Soil metabolic processes are determined by enzymes whose effects are influenced by physical, chemical, microbiological, and biochemical aspects (Das & Varma, 2010). Extreme weather fluctuations and improper soil management impact the level and type of soil enzymatic activity. Reduction in enzyme activity can negatively impact ecosystem services such as nutrient cycling, decomposition, and plant-microbe interactions, affecting productivity and net C balance (Roa *et al.,* 2017). Amylase, phosphatase, cellulose, ß-glucosidase, chitinase, arylsulfatase, dehydrogenase, and urease are just a few of the enzymes found in soil that plants, animals, and microorganisms can release.

In the biochemical process of recycling organic matter in the soil system, soil enzymes play a significant role. The biological-biochemical characteristics of soil have received increasing attention recently since they are more sensitive to small changes in soil management techniques and land use changes (Rao *et al.,* 2017). Finding a shift in the overall microbial population of a specific soil might be considered a sensitive sign of a change in the soil health state (Karaca *et al.,* 2010). Ion exchange, entrapment, copolymer synthesis, adsorption, cross-linking, microencapsulation, and covalent attachment are all examples of enzyme states that are found in soil. Free enzymes that enter the soil system can either combine with humic colloids or become stabilised by clay surfaces and organic matter (Karaca *et al.,* 2010).

No-tillage, residue retention, and crop rotation have been demonstrated to increase enzymatic activity (Hok *et al.,* 2018; Muzangwa *et al.,* 2020). Fluorescein diacetate (FDA) hydrolase and ß-glucosidase are two soil enzymes that catalyse the break down complex organic molecules and are linked with fungal and microbial biomass. These enzymes' activity has been frequently employed as indicators of how soil microbial populations respond to various environmental stressors (Pittarello *et al.,* 2022). Although soil enzymes are not part of the Biofunctool®, numerous studies (Hok *et al.* 2018; Rao *et al.,* 2017; Saccá *et al.,* 2017) have demonstrated their significant role in soil ecosystem functioning. The functions of soil enzymes are closely linked to the physical properties of the soil, SOM, microbial activity and/or biomass of residues (Rao *et al.,* 2017).

Soil enzymes have proven effective soil health indicators across various agricultural systems. It is vital to understand how soil enzymes function and the factors that impact their activity to

enhance soil management, soil health, and food production. Soil enzymes play a key role in catalysing and facilitating decomposition and nutrient cycling, making them a valuable biological soil health gauge. It is worth noting that the activity of a single enzyme alone cannot provide a comprehensive snapshot of soil health, as each enzyme may only catalyse a specific reaction. Therefore, to effectively gauge soil quality, it's necessary to assess multiple enzyme activities simultaneously (Adetunji *et al.,* 2017). Integrating soil enzymes with other indicators can provide a more comprehensive soil health assessment.

2.5.1 *ß-glucosidase*

Glucosidase is a widespread enzyme found in soils. It catalyses the biodegradation and hydrolysis of various ß-glucosidase, which is found in decomposing plant debris (Das & Varma, 2010). The enzyme is also involved in the saccharification of cellulose (Karaca *et al.,* 2010). ß-glucosidase is a key enzyme in the soil's transformation and breakdown of organic materials. It creates glucose, which is essential for soil microbes as a source of carbon energy (Riah *et al.,* 2014). ß-Glucosidase enzymes release the measured end products in quantifying the synergistic activity of enzyme systems that hydrolyze complex carbohydrates (Deng & Popova 2011). Carbohydrates have a crucial function in sustaining microbial life as carbon energy and energy sources in soil, according to Deng & Popova (2011). Enzymes that hydrolyze carbohydrates are necessary for the breakdown, especially cellulose and mineralization of organic compounds, but also the emergence and development of soil organic matter and structural components.

Compared to other soil health indicators, ß-glycosidase responds to changes in soil management more rapidly (Hok *et al.,* 2018). A study by Hok *et al*. (2018) discovered that at a depth of 0–5 cm, ß-glycosidase was significantly greater in a no-tilled system with crop retention than in a traditional system. The higher ß-glycosidase rates might be accounted for by crop residues adding carbon to the soils, which might increase the enzyme's activity. A study done by Muzangwa *et al.* (2020) also indicated that ß-glycosidase significantly improved under residue retention.

2.5.2 *Fluorescein diacetate assay (FDA)*

Fluorescein diacetate is a nonpolar compound that can be used to measure microbial activity. It can diffuse easily through cell membranes, where it can be hydrolyzed and release fluorescein (Prosser, 2011). The soil environment is rich in the enzymes necessary for FDA hydrolysis. Various organic tissues are broken down by non-specific lipases, esterases, and

proteases that have been found to hydrolyze FDA. Thus, many organisms can hydrolyze FDA, particularly the primary decomposers, bacteria and fungi (Adam & Duncan, 2001). Therefore, FDA is a reliable indicator of overall microbial activity and a measurement of SOM turnover. Plant residue and crop rotation both increase the FDA measurement. A fallow time between maize harvests was discovered to dramatically lower FDA results than a system that included crop rotations (Muzangwa *et al.,* 2020). A recent study done by Pittarello *et al.* (2022) highlighted how FDA was much higher under a CA system than under a conventional system, indicating a higher microbial community.

2.6 Conclusion

Preserving the natural resources capable of supporting agricultural systems is imperative to sustain rather than merely meet the food production demands. It has also become apparent that crop production systems should respond to climate change. Ecosystem services like C storage in the soil, effective nutrient cycling, climate regulation and biodiversity preservation can be provided by CA systems that depend on the diversity of animals, plants, and soil microorganisms at various levels (Sanderson *et al.,* 2013). However, there are challenges associated with the application of CA, even though research has demonstrated its significance. Conservation agriculture is site-specific and depends on the regional socioeconomic and biophysical environments. Hence, there are numerous forms and applications of CA, each with unique needs regarding labour, supplies, fertiliser, etc. (Giller *et al.,* 2009). Testing soil health can provide valuable information for making informed decisions about soil management unique to a set of pedoclimatic conditions is important.

Considering all aspects of the soil's health in soil health assessments is necessary; however, it is not always feasible because of the time and expenses involved. Thus, a complete soil health index can be obtained by combining soil enzymes with the Biofunctool® index to create new, quick, and simple means of analysing the overall soil health. Understanding the potential functions of soil enzymes and the soil's overall structure, nutrient cycle, and carbon transformation is crucial for preserving the health of the soil and managing its fertility in agricultural ecosystems. These activities may significantly affect ecosystems' soil biology, environmental regulation, plant development, and nutrient uptake (Das & Varma, 2010). By assessing the soil health of farmers' CA systems in different agroecosystem regions using the Biofunctool® index and the activity of selected soil enzymes can aid in educating policy-makers and development professionals about the detrimental effects of existing agricultural

practices and highlights the benefits of alternative growing techniques like CA on the overall soil health (Pheap *et al*., 2019).

Chapter 3: Materials and Methods

3.1 Study areas

The study was carried out in two municipality districts in the provinces of the North-West and the Free-State in South Africa. The sampling points in the North-West Province were taken around the town of Ottosdal in the Ngaka Modiri Molema municipality district (Figure 3.1). In the Free-State Province, the sampling sites were Clocolan and Ladybrand (Figure 3.1), and both towns are located in the Thabo Mofutsanyana municipality district.

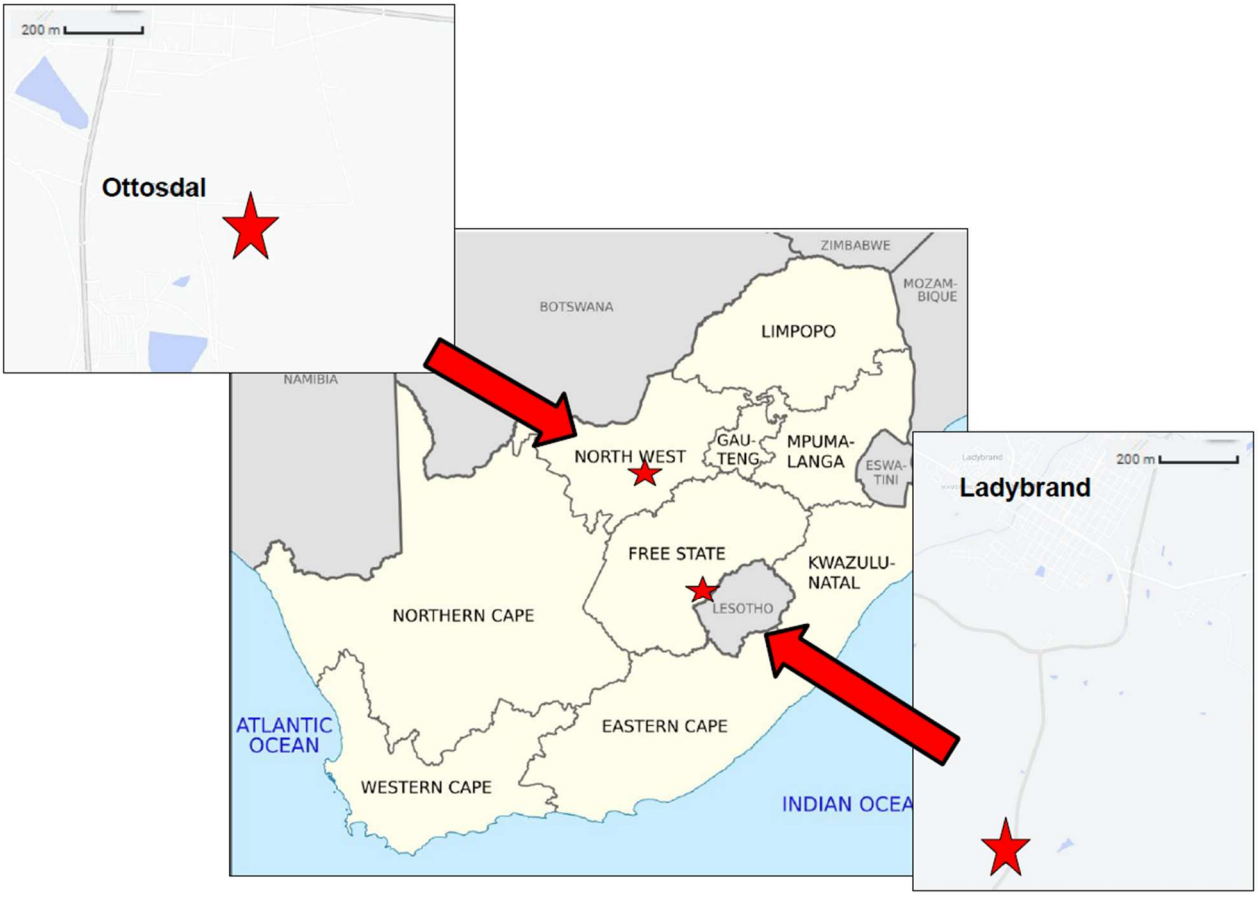


Figure 3.1: Location of the two study sites in the North-West Province and Free-State province indicating the nearest towns to each study location (red star indicated study locality) (Google Maps, 2023).

3.2 Pedo-climatic context of the study areas

3.2.1 *Climate and biomes*

The two research areas are in the grassland biome (Mucina & Rutherford, 2010). The grassland biome is a distinct ecological region characterized by vast expanses of grasses with relatively few trees and shrubs. This biome is known to be used for agriculture and is located

in a semi-arid environment (Mucina & Rutherford, 2010). The climate of the Ottosdal in Ngaka Modiri Molema district is characterised by long, warm summers and brief, cold, and arid winters. Throughout the year, the temperatures can range from 3 to 30°C. The typical wet season runs from October to April, with January often receiving the most rainfall. May through September are the dry months, with July being the driest month (WeatherSpark, 2022a). Like Ottosdal, the research areas in Thabo Mofutsanyana district have warm summers, and winters are brief, cold and dry. The temperature can vary across the year from -2 to 31°C. Rain seasons are the same as in the North-West (WeatherSpark, 2022b). The average rainfall of the two districts ranges, as can be seen in Table 3.1. The average altitude in Ottosdal is 1359 m, whilst that of the Ladybrand and Clocolan is 1588 m above sea level.

Table 3.1: Monthly rainfall range figures from the Ngaka Modiri Molema and the Thabo Mofutsanyana district from July 2021 to August 2022. Data retrieved from The South African Weather Service, historical rain maps (2022).

	Thabo Mofutsanyana district	**Ngaka Modiri Molema district**
Month	**Rainfall (mm)**	**Rainfall (mm)**
July 2021	0-10	0-10
August 2021	0-10	10-25
September 2021	0-10	10-25
October 2021	50-100	10-25
November 2021	50-100	25-50
December 2021	100-200	100-200
January 2022	100-200	50-100
February 2022	50-100	25-50
March 2022	50-100	50-100
April 2022	100-200	100-200
May 2022	10-25	10-25
June 2022	0-10	10-25
July 2022	0-10	0-10
August 2022	0-10	0-10
September 2022	0-10	0-10
October 2022	0-10	0-10
November 2022	50-100	50-100
December 2022	10-25	10-25
January 2023	10-25	10-25
February 2023	50-100	25-50
March 2023	50-100	50-100

3.2.2 *Geology and soils*

The Dominion Group dominates the Ngaka Modiri Molema district and is a volcano-sedimentary sequence of the Archaean age underlying the Witwatersrand Supergroup. In the Ottosdal area, this group is comprised of clastic sediments, felsic volcanic, and subordinate mafic lavas (Jackson, 1992). The soils observed in the sampling locations in this district are ferric lixisols and chromic cambisols (Soil Atlas of Africa, 2013). This information is summarised in Table 3.2. Lixisols can be described as soils with pedogenetic clay differentiation. Clay content tends to be less in the topsoil than in the subsoil. These soils are derived from a wide variety of parent materials, notably unconsolidated, chemically strongly weathered, fine-textured materials. Cambisols are characterised by slight or moderate weathering of parent material and by the absence of appreciable quantities of illuviated clay, organic matter, Al and/or Fe compounds (IUSS Working Group, 2015).

On the other hand, the Thabo Mofutsanyana district is part of the Karoo-Supergroup and can further be divided into the Stormberg Group and the Molteno Formation. The Molteno Formation contains alternating finely-grained, medium-grained, coarse-grained sandstones and greyish mudstone layers (Bordy *et al*., 2005). The soils and geology of this district are summarised in Table 3.2. The Free-State soils consist of ferric lixisols, eutric planosols and haplic arenosols. Planosols are soils with a mostly light-coloured horizon showing signs of periodic water stagnation and abruptly overlies a dense, slowly permeable subsoil with significantly more clay. They are derived mostly from alluvial and colluvial deposits. Arenosols are comprised of deep sandy soils, including the soils in residual sands, after in situ weathering of usually quartz-rich sediments or rock (IUSS Working Group, 2015).

The soil's texture class is also recognized and can significantly impact soil management. The soils in both districts mainly consist of loamy sand, sandy loam and sandy clay loam. Loamy sand contains 70 to 85 % sand, up to 30 % silt, and up to 15 % clay. Sandy loam consists of either 20% clay or less, with the combined percentage of silt and twice the clay percentage exceeding 30% and comprising 52% or more sand. Alternatively, it can contain less than 7% clay, less than 50% silt, and between 43% and 52% sand. A sandy clay loam is a soil material that contains 20 to 35 % clay, less than 28 % silt, and 45 % or more sand (Lawinsider, 2022).

Table 3.2: Summary of each study district's dominant soil type, texture and geology.

District	GPS Coordinates	Soil type	Soil texture	Geology
Ngaka Modiri Molema	S26° 46' 37.617" E25° 54' 56.0514"	Ferric Lixisols, Chromic cambisols	Loamy Sand, Sandy clay loam	Dominion Group
Thabo Mofutsanyana	S29° 14' 42.4026" E27° 25' 39.7452"	Ferric Lixisols, Haplic Arenosols	Loamy sand, Sandy loam	Molteno Formation

3.3 Treatment description

The two study sites are approximately 383 km from each other, and the sampling locations are spread over farms owned by landowners with differing agricultural practices. All study sites included conservation agriculture (CA), conventional agriculture (CT), and uncultivated fields (Un). The entire study was grounded in a survey conducted with farmers from the designated fields. Farmers who had been applying Conservation Agriculture (CA) techniques for over five years were selected for CA fields, and adjacent uncultivated fields, where no crops were ever planted and natural grasses were growing, were also considered. Ngaka Modiri Molema district had 8 CA, 1 CT and 3 uncultivated fields. In contrast, the Thabo Mofutsanyana district had 9 CA, 6 CT and 7 uncultivated fields. Table 3.3 summarises the total sites sampled, and Table 3.4 summarises the number of sites grazed and not grazed. Major crop types for both sampling locations were Sunflower (*Helianthus annuus*), Soybean (*Glycine max*) and Maize (Zea mays), with some of the CA farmers planting cover crop mixtures in rotation with cash crops.

Table 3.3: A summary of the number of farming systems across the two districts

District	Conservation agriculture (CA)	Conventional agriculture (CT)	Uncultivated (UN)
Ngaka Modiri Molema	8	1	3
Thabo Mofutsanyana	9	6	7

Table 3.4: A summary of the grazed and non-grazed sites across the two districts

District	Grazed Ca	Grazed CT	Grazed UN	Non-grazed Ca	Non-grazed CT	Non-grazed UN
Ngaka Modiri Molema	3	0	2	5	1	1
Thabo Mofutsanyana district	7	4	6	2	2	1

3.4 Data collection

3.4.1 *Sampling*

Data collection was carried out using soil sampling and laboratory analysis. Soil sampling for enzyme and active carbon analysis was done in August 2021, February 2022, August 2022, and February 2023 to capture the seasonal variations. However, the sampling of the Biofunctool® was done in the latter two sampling periods since training in the Biofunctool® only took place in May 2022. Furthermore, the soil sampling for the Biofunctool® made use of fewer sampling sites to match the available resources (Table 3.5). Samples for enzyme and active carbon measurements were collected by mixing five random soil samples at 10 cm depth to create a composite sample at each sampling site. Samples were returned to the North-West University in Potchefstroom, air-dried for two weeks, and sieved through a 2mm sieve before further laboratory analyses. Sampling for Biofunctool® parameters was carried out in three replicates for each site. Further details regarding the sampling for each Biofunctool® parameter are given in section 3.5.

Table 3.5: A summary of the number of farming systems sampled for the Biofunctool® across the two districts

District	Conservation agriculture (CA)	Conventional agriculture (CT)	Uncultivated (UN)
Ngaka Modiri Molema	2	1	2
Thabo Mofutsanyana	2	2	2

3.5 Biofunctool® parameters

3.5.1 *Aggregate stability*

This technique entailed gathering aggregates in the field and letting them dry in the sun for an hour. For the 0–2 cm soils (AggSurf) and the 2–10 cm soils (AggSoil), six soil aggregates of approximately 6–8 mm diameter were collected at each sampling location, resulting in a final total of 18 aggregates for one depth. The samples were taken back to the North-West laboratory, Potchefstroom, where the aggregates were air-dried until use. The dried aggregates were put into the tiny sieves, with a mesh size of 1mm (Figure 3.2A). Water was put into a flat plastic box to a depth of 1 cm. One sieve was immersed every 15 seconds until all 18 sieves were in the water (Figure 3.2B). The samples were scored based on the time to slaking during the first five minutes. Depending on the aggregate stability, a score of 0 to 3 was assigned to each sieve, following the criteria in Table 3.6. After five minutes, when some of the aggregates were still intact, each sieve was then raised and lowered in a 2-second

rhythm. Depending on how much soil is still in the sieve, a score of 4, 5 or 6 was assigned (Herrick *et al*., 2001). After all samples were tested an average score was then calculated for AggSurf and AggSoil.

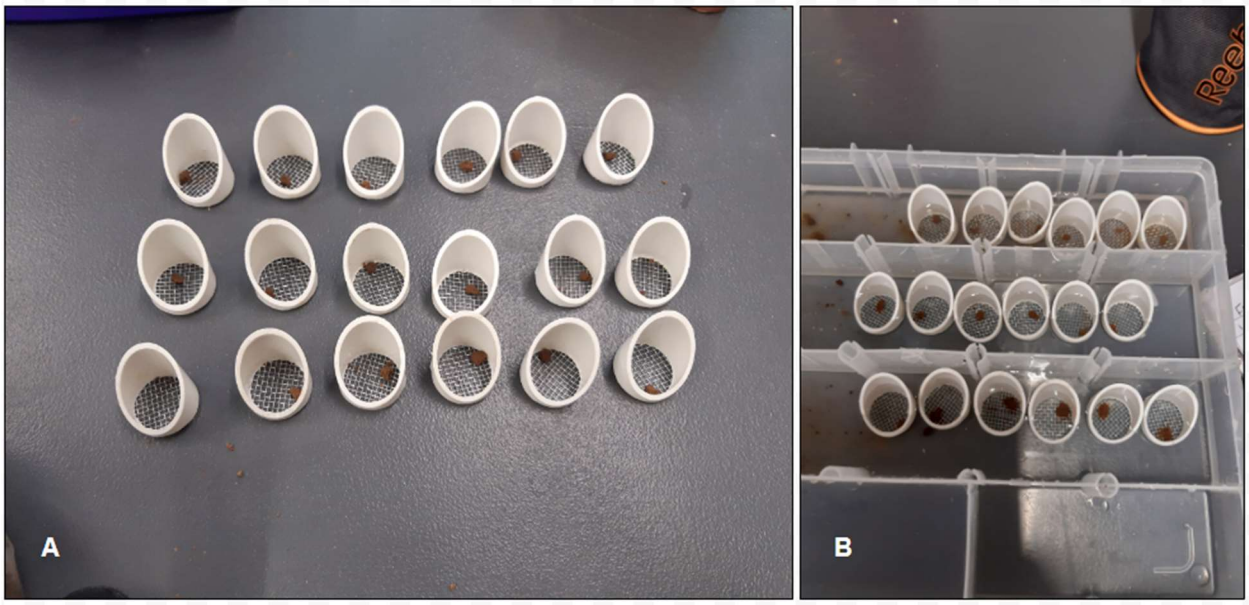


Figure 3.2: A) Soil aggregates before immersed in water. **B)** Soil aggregates after immersion in water (Photos taken by Anize Tempel, NWU).

Table 3.6: Criteria for the assignment of aggregates to stability classes (Herrick *et al*., 2001)

Stability Class	Criteria for assignment to stability class (For standard characterization)
0	Soil is to unstable sample (falls through sieve)
1	50% of structural integrity lost within 5 s of insertion into water
2	50% of structural integrity lost 5-30 s after insertion
3	50% of structural integrity lost 30-300 s after insertion or < 10% of soil remains on sieve after 5 dipping cycles
4	10-25% of soil remains on sieve after 5 dipping cycles
5	25-75% of soil remains on sieve after 5 dipping cycles
6	75-100% of soil remains on sieve after 5 dipping cycles

3.5.2 *Visual evaluation of soil structure*

This approach categorizes the soil structure of each layer into five scoring classes, providing a rapid assessment of how soil structure aligns with biological communities in the field. A spade was used to dig a 25x25x25 cm hole at each location to reveal the soil strata. An example of how the layer was exposed is seen in Figure 3.3. The chart shown in Figure 3.4

was used to separate the layers by assessing the aggregates, checking roots and identifying the colour change of the soil horizons (Guimarães *et al*., 2011).

Figure 3.3: Exposure of soil layers in the field during the measurement of VESS (Photo: Anize Tempel, NWU).

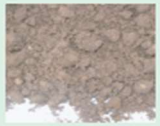
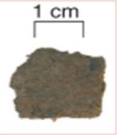
Structure quality	Size and appearance of aggregates	Visible porosity and Roots	Appearance after break-up: various soils	Appearance after break-up: same soil different tillage	Distinguishing feature	Appearance and description of natural or reduced fragment of ~ 1.5 cm diameter
Sq1 Friable Aggregates readily crumble with fingers	Mostly < 6 mm after crumbling	Highly porous Roots throughout the soil			 Fine aggregates	 The action of breaking the block is enough to reveal them. Large aggregates are composed of smaller ones, held by roots.
Sq2 Intact Aggregates easy to break with one hand	A mixture of porous, rounded aggregates from 2 mm –7 cm. No clods present	Most aggregates are porous Roots throughout the soil			 High aggregate porosity	 Aggregates when obtained are rounded, very fragile, crumble very easily and are highly porous.
Sq3 Firm Most aggregates break with one hand	A mixture of porous aggregates from 2 mm –10 cm; less than 30% are <1 cm. Some angular, non-porous aggregates (clods) may be present	Macropores and cracks present. Porosity and roots both within aggregates.			 Low aggregate porosity	 Aggregate fragments are fairly easy to obtain. They have few visible pores and are rounded. Roots usually grow through the aggregates.
Sq4 Compact Requires considerable effort to break aggregates with one hand	Mostly large > 10 cm and sub-angular non-porous; horizontal/platy also possible; less than 30% are <7 cm	Few macropores and cracks All roots are clustered in macropores and around aggregates			 Distinct macropores	 Aggregate fragments are easy to obtain when soil is wet, in cube shapes which are very sharp-edged and show cracks internally.
Sq5 Very compact Difficult to break up	Mostly large > 10 cm, very few < 7 cm, angular and non-porous	Very low porosity. Macropores may be present. May contain anaerobic zones. Few roots, if any, and restricted to cracks			 Grey-blue colour	 Aggregate fragments are easy to obtain when soil is wet, although considerable force may be needed. No pores or cracks are visible usually.

Figure 3.4: Visual evaluation of soil structure chart used for in-field analyses of soil structure (Guimarães *et al*., 2011).

3.5.3 *Beerkan test*

A plastic cylinder 20 cm in diameter was inserted about 1cm into the ground of a field to be sampled using a piece of wood and a hammer (Figure 3.5). Any litter within the cylinder zone was meticulously removed. Ten bottles, totalling 310 ml of water, were filled. A piece of plastic was placed inside the cylinder to prevent a splash effect and soil disturbance when pouring the water. The stopwatch started once one bottle of water had been poured into the cylinder. Following the first bottle, the second one was poured as soon as the water had finished filtering, and the time was recorded. This process was repeated until all ten bottles had been emptied or 30 minutes had gone (Lassabatère *et al.,* 2006).

Figure 3.5: A 20 cm (diameter) cylinder was placed into the soil at a depth of 1 cm to measure water infiltration using the 310 ml filled water bottles (Photo: Anize Tempel, NWU).

The finale water infiltration rate was determined by calculating the time into a two decimal number expressed in a minute. Using Excel, the cumulated volume was expressed as a function of cumulative time. The slope of the regression of the permanent phase corresponds to the water infiltration rate in ml/min.

3.5.4 *Soil available nitrogen*

Samples collected from the field were sent to Intertek lab for available nitrogen analyses. Samples for nitrogen analyses were kept cool and protected from direct sunlight until they reached the laboratory.The inorganic nitrogen was extracted using 1M KCl solution, as described by Maynard *et al.* (1993). About 50 grams of dried sieved soil was added to 180ml of 1M KCl solution and shaken for 1 hour on an automatic horizontal shaker. The solution was filtered through a Whatman No.2 ashless filter paper. The filtrate was then tested on an auto analyser for NO_3^- and NH_4^+.

3.5.5 *Ion exchange membrane*

The method employs an exchangeable membrane capable of efficiently absorbing nutrients from a solution, allowing for the assessment of the dynamics of readily available nutrients within the soil. Only anion exchange membranes (VWR chemicals) were used for this experiment. The anion membranes were prepared by placing them on a glass slab with some distilled water to prevent the membranes from drying out before cutting them into sizes of 6 cm in length and 2.5 cm in width. A tiny hole was made on each anion leaf using a needle, and a fishing line was tied to make it easy to locate the membranes in the soil. The membranes were placed in a 0.5M $NaHCO_3$ solution and kept cool until transferred to the field.

The membranes were transported to the field in cooler bags containing ice. One membrane was placed horizontally about 8 cm deep into the soil and covered with the soil (Figure 3.6). The fishing line was then wrapped around a stake to simplify locating the membranes on collection. After two weeks, the membranes were gently removed from the soil and placed in falcon tubes with 35 ml of a 1M KCl solution. These tubes were returned to the lab and put on an automatic shaker for 16 hours at 100 rpm before filtering through a Whatman No.2 paper. Filtrates were stored in the freezer until further testing (Qian & Schoenau, 2002). The falcon tubes were taken to Intertek lab, where the filtrate was tested using an auto-analyzer for NH_4^+ and NO_3^-.

Figure 3.6: A) Membrane put into the soil. **B)** Membrane removed after 2 weeks in the soil (Photo: Dimakatjo Mmotla, NWU).

3.5.6 *Active Carbon/Permanganate oxidizable carbon (POXC)*

Active carbon constitutes a segment of soil organic matter (SOM) readily available as a source of sustenance and energy for the soil microbial community. The quantification involves employing the permanganate-oxidizable carbon (POXC) method, a procedure outlined by Weil *et al.* (2003). For each sample, 2.5 g of air-dried soil was weighed into two 18 ml distilled water falcon tubes. Then, 2 ml of 0.2M stock solution of potassium permanganate ($KMnO_4$) stock solution was added to start the redox reaction. The falcon tubes were capped and placed on an automatic shaker for two minutes at 120 rpm. The tubes were taken out of the shaker, uncapped, and placed on a bench for eight more minutes. Two extra falcon tubes were filled with 49.5 ml of distilled water and received 0.5 ml from the 18 ml falcon tubes to stop the reaction through dilution, as seen in Figure 3.7, before closing them and manually shaking them for 10 seconds. The absorbance of each diluted sample was measured using a spectrometer at a wavelength of 550 nm. When there was more than 5% difference in absorbance, duplicates were repeated (Weil *et al.*, 2003). Standards with 0.005M, 0.01M, and 0.02M concentrations of the 0.2M stock solution were prepared. The standard curve was calculated using the following equation below:

$$\text{Concentration} = a + b * \text{absorbance}$$

Where the concentration of POXC is the sum of the slope (b) and y-intercept (a) of the linear regression equation.

Figure 3.7: Falcon tubes containing 49.5 ml of dilution (far left and far right). The middle falcon tubes contain 18 ml distilled water, 2.5 g soil, and 2 ml $KMnO_4$ (Photo: Anize Tempel, NWU)

The POXC was calculated using the following equation below:

$$\text{Active C (mg/kg)} = [0.02\text{mol/L (a+b * absorbance)}] * (9000\text{mg C/mol}) * (0.02\text{L solution}/0.0025\text{kg soil}).$$

1. 0.02 mol/L is the initial $KMnO_4$ concentration.
2. 9000 mg is the amount of carbon (0.75mol) oxidised by 1 mol of $KMnO_4$.
3. 0.02 L is the volume of the $KMnO_4$ solution.
4. 0.0025 kg is the amount of soil weighed.

3.5.7 *Basal soil respiration (SituResp)*

A soil sampling cylinder was used in the field to collect roughly 150 g of soil, which was then placed in a cool box with ice packs until basal soil respiration determination. A 5 mm sieve was used to sieve the soil at the lab, and 100 g of the sieved soil was placed in an airtight jar. Plastic cuvettes were filled with the warm (60-65°C) indicator solution containing 16.77 g of KCl, 0.315 g of $NaHCO_3$, and 0.0187 g of Cresol red. The solution cooled down to gel, and the cuvette's absorbance was read on a spectrophotometer at a wavelength of 570nm. The

cuvettes with a known absorbance were placed in each jar with the soil after 24 hours at 25°C in a soil oven, as seen in Figure 3.8. The cuvette's absorbance was measured once more at a wavelength of 570 nm following the 24-hour period (Thoumazeau *et al*., 2019). The data was calculated following the equation below:

$$SituResp = AbsT0 - AbsT24$$

ABST0 - Absorbance of solution in cuvette
BST24 - Absorbance of solution in cuvette after 24 hours

In order to make the indicator solution, 1.5g of Agar was mixed with 50 ml of water using a hotplate and magnetic stirrer, and the mixture had to reach 90°C. When the solution reached 90°C, it was diluted with another 100 ml of water and kept at a constant temperature of 60-65°C. At this constant temperature, 1.5 ml of the solution was poured into plastic cuvettes and left on a bench to cool down. For one week, the cuvettes were placed in a desiccator at room temperature, in the dark, with an excess of 200 ml of 1M NaOH. Over the course of a week, the gel's colour changed from red to purple.

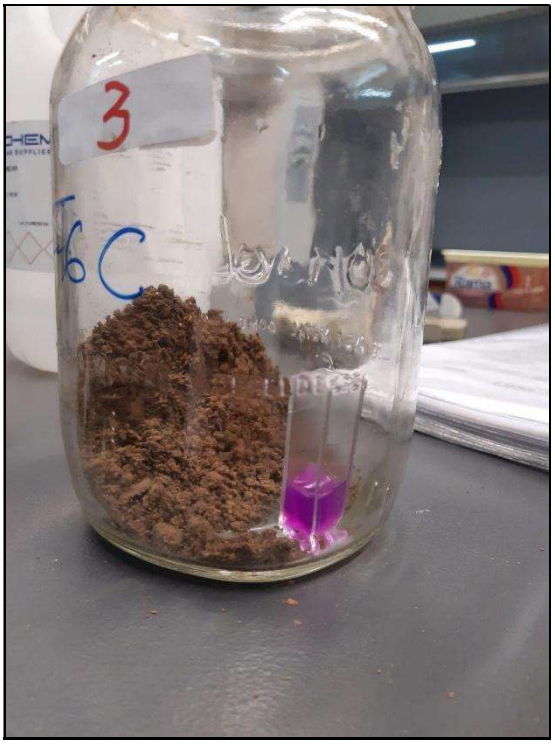


Figure 3.8: The 100 g of soil with a cuvette filled with the indicator solution (Photo taken by Anize Tempel, NWU).

3.5.8 *Bait lamina*

The lamina baits were made according to Kratz's procedures (2016). Each lamina bait was made of a PVC strip, measuring 120 mm in length, 6 mm in width and 1 mm in thickness, with 16 holes. The holes on each of the lamina strips were filled with a substrate made of agar, cellulose, wheat, and activated charcoal. The substrate was made by dissolving 1 g of agar in boiling water and adding drop by drop 5 g of cellulose, 5 g of flour, and 2.5 g of active charcoal, creating a homogeneous paste. The holes were filled by pulling the strips between the thumb and finger with the paste. After the first layer had been air-dried for 24 hours, the second coat was added to ensure all the holes were filled. A total of 8 bait lamina strips, replicated three times, were inserted at each sampling location. They were spaced, vertically, 30 cm apart and placed in a straight line (Figure 3.9). The sites were marked with hazard tape for quick identification. After about two weeks, the lamina strips were removed, and each hole of the bait lamina strips received a score based on the state of deterioration. The scoring criteria used are presented in Table 3.7 (Gestel *et al*., 2003).

Table 3.7: The score attributed to each hole depended on the extent of the degradation of the holes on the lamina strip.

State of the degradation	**No degradation**	**Partial degradation**	**Full degradation**
Score for analyses	0	0.5	1

Figure 3.9: The process of adding the bait lamina sticks into the soil (Photo: Dimakatjo Mmotla, NWU).

The following equation was used:

Score for each lamina (a) (%deg.d^{-1}) = Average (16 holes of each lamina)/ Incubation time (Day)

Lamina (%deg.d^{-1}) = Average (8 Scores for the 8 lamina (a) per sampling point)

3.6 Soil organic carbon and enzymes

3.6.1 *Soil organic carbon*

Soils for soil organic carbon (SOC) were taken to Intertek lab for analysis. The determination of SOC was based on the Walkley-Black chromic acid wet oxidation method. Oxidisable matter in the soil was oxidised by 1 N dichromate ($K_2Cr_2O_7$) solution. The reaction was assisted by the heat generated when two volumes of sulphuric acid (H_2SO_4) were mixed with one volume of the dichromate. The remaining dichromate was titrated with ferrous sulphate ($FeSO_4$). The titre is inversely related to the amount of C present in the soil sample (Allison, 1965).

The following equation was used to determine the percentage of SOC:

$$SOC\% = \frac{[mlFe(NH_4)_2(SO_4)_2 blank - mlFe(NH_4)_2(SO_4)_2 sample]\ x\ M\ x\ 0.3\ x\ f}{soil\ mass\ (g)}$$

Where M refers to the concentration of Fe (NH4)2 (SO4)2 in mol mL-1

3.6.2 *Fluorescein diacetate hydrolysis (FDA) analyses*

Fluorescein diacetate hydrolysis was analysed following the method described by Prosser *et al.* (2011). A gram of soil for each sample was weighed and added to 250 ml glass bottles. Each sample had two replicates and an additional 1 g of soil that was weighed for the control. Each glass bottle received 0.50 ml of the FDA substrate and 50 ml of THAM buffer. Only 0.5 ml of acetone was added to the control bottles. The glass bottles were then covered with caps, well mixed, and placed in an incubator set at 37°C for three hours. Additionally, a blank without any soil was created using only the FDA substrate and THAM buffer. After three hours, the samples were taken out of the oven, and to stop the FDA hydrolysis, 2 ml of acetone was added to each bottle and stirred. Figure 3.10 illustrates the changing of colour after three hours in the incubator. After three hours, a Whatman No. 2 filter paper was used to filter the samples. The filtrate was put into a cuvette to measure the absorbance at a 490 nm wavelength using a spectrophotometer (LLG-*uni*SPEC 2, LLG Labware). A standard containing fluorescein

($C_{20}H_{12}O_5$) was made with the following concentrations: 0.03M, 0.1M, 0.3M, and 0.5M (Prosser *et al.* 2011). The fluorescence activity was calculated using the equation below:

$$FDA\ hydrolic\ activity\left((mg fluorescein\ kg^{-1}hr^{-1}) = \left[\frac{(S-C)}{DM}/3\right]\right)$$

Where S is the concentration of fluorescein (mg) in the sample, C is the concentration of fluorescein (mg) in the control, and DM is the dry mass of soil aliquot (Prosser *et al*. 2011).

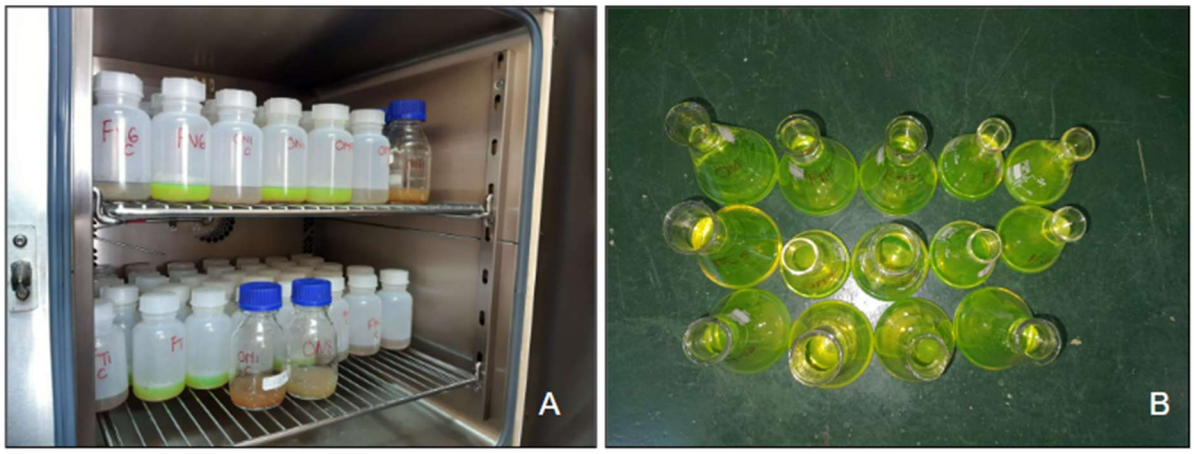


Figure 3.10: A) FDA solution after 3 hours in the oven. Note that the control bottles have not changed colour. **B)** FDA solution after being filtrated (Photo: Anize Tempel, NWU).

3.6.3 *β-glucosidase analyses*

The activity of *β*-glucosidase was measured by the *p*-nitrophenol generated when 1 g of soil was incubated with a p-nitrophenyl substrate at the ideal pH for the enzymatic reaction (Deng & Popova, 2015). The first step in measuring β-glucosidase was to make the modified universal buffer (MUB). Following the preparation of all solutions, 1 g of soil for each soil sample and an additional 1 g for a control was measured. Toluene was added to the measured soil samples and left under a fume hood for approximately 15 minutes. Thereafter, 1 ml of the PNG solution was added only to the main soil bottles and not the control bottles and 4 ml of the MUB solution was added to all soil samples. The bottles were then incubated at 37°C for one hour. The caps were removed, 1 ml of 0.5M $CaCl_2$ and 4 ml of THAM pH 12 were added to each sample, and they were all thoroughly mixed (Figure 3.11). The THAM pH 12 buffer serves to stop the enzymes' activity. The 1 ml PNG was added to the control flasks at this point. The soils were then filtered using a Whatman No. 2V folded filter paper. The intensity of the filtrate's yellow colour was then measured at 405 nm using a spectrophotometer. *P*-

nitrophenol release was measured in accordance with a calibration curve created using standards.

Figure 3.11: A) The *β*-glucosidase solutions after they have been incubated for an hour. **B)** The process of filtering the substrate after the enzyme activity has been stopped (Photo:Anize Tempel & Dimakatjo Mmotla, NWU).

3.7 Statistical analyses

3.7.1 *Statistics on soil variables*

Permutational multivariate analysis of variance (PERMANOVA) was performed on the Biofunctool® indicators using R software (R Development Core Team, 2008). Linear models were used to investigate meaningful and significant correlations on the effects of the farming systems and season on the Biofunctool® indicators. For each linear model, each factor's contributions to the model, overall were analysed by dividing the sum of squares related to the considered factor using the R^2 coefficient of determination. After separately analysing the variables, a multivariate analysis was performed using principal component analysis (PCA). Weights were assigned to each Biofunctool® indicator to consider each of the three soil functions equally (physical, chemical, and biological). The calculations are discussed in section 3.7.2.

Data on the soil variables (Active Carbon, FDA, β-glucosidase and SOC) from the study sites were transformed [log (x+1)] and normalised before being analysed in a permutational multivariate analysis of variance (PERMANOVA). The study examined the impact of sampling times, which were fixed at four levels (August 2021, March 2022, August 2022, and February 2023), farming systems at three fixed levels (conventional, conservational, and uncultivated), and grazing at two fixed levels (grazed and non-grazed), as well as their interactions, using a multifactorial approach. In the presence of significant interactions, variables were assessed individually through pair-wise comparisons in the same design as above. A correction was applied for the 5 % significance level to control the family-wise error rate produced by a high number of tests (Wheldon *et al*., 2007). Data was ordered through non-metric multidimensional scaling (MDS) of centroids from each factor of interest. Regression analyses were performed between biotic variables and the Biofunctool® ones. All tests were done at a 5% significance level.

3.7.2 *Calculation of soil quality indices*

A soil quality index (SQI) was calculated following the procedure described by Thoumazeau *et al.* (2019b). The SQI was derived from the weightings from multivariate analyses, in this case, the principal component analysis (PCA). The SQI was calculated according to equations (1) and (2), with a weighting of the transformed variables using the PCA eigenvectors and the percentages of total variability explained by each principal component:

$$\mathrm{Wi} = \sum_{j=1}^{p} \lambda \mathrm{jxfj} \qquad (1)$$

$$\mathrm{SQI} = \sum_{i=1}^{n} \mathrm{SixWi} \qquad (2)$$

Fij = relative percentage of total variability attributed to each PC.

j = sum of squared coordinates on each eigenvector.

Si = Normalised indicator scores

Wi = weighted factors

Chapter 4: Results

The upcoming chapter will delve into the outcomes derived from this research. It will commence by reporting the results of the Biofunctool® indicators, examining both univariate and multivariate analyses. The chapter will then explore the soil quality index obtained from these Biofunctool® indicators. Subsequently, the presentation of the results will shift to enzymes, active carbon, and soil organic carbon. Finally, the chapter will conclude by investigating potential correlations between the enzyme data and the Biofunctool® indicators.

4.1 Impact of farming system, grazing, and sampling time on the Biofunctool® Indicators

The PERMANOVA analysis to assess the main effects and interactions of farming systems, grazing, and sampling time (season) on the Biofunctool® parameters did not reveal, except for a few (Table 4.1), significant differences ($P > 0.05$) for most parameters. Notably, the season, represented by the sampling time, significantly impacted Lamina and SituResp ($P < 0.0001$) (Table 4.2). Conversely, the farming system significantly influenced AggSurf, AggSoil, active carbon and Situresp (Table 4.1 & 4.2). Tables 4.3 and 4.4 provide an overview of the trends observed in the Biofunctool® parameters based on their respective ecosystem function over the August 2022 and February 2023 sampling periods.

4.1.1 *Structure maintenance*

The results from the structure maintenance parameters showed reduced soil structure stability under the conventional system with a VESS score of 1.67 (Tables 4.3 & 4.4). The conventional agriculture system had lower values of the AggSurf and AggSoil scores and, although not significant, a much lower infiltration rate compared to the conservation agriculture system and uncultivated fields (Tables 4.3 & 4.4). The conventional system had much lower scores for AggSurf, 2.2 for August 2022 and 1.92 for February 2023. Higher scores of AggSurf and AggSoil for the conservation and uncultivated systems were evident. From the PERMANOVA table (Table 4.1), farming systems had a significant influence on the aggregate stability ($P<0.05$). In the February 2023 season, the infiltration rates for the uncultivated field were significantly lower at 39.76 ml/m compared to August 2022, where rates reached 117.3 ml/m.

Table 4.1: PERMANOVA results of measured Biofunctool® soil indicators across two sampling seasons and sites (n=33). Ns for p > 0.05, *p < 0.05, **p < 0.01, ***p < 0.001.

		Measured variable	Sampling time		Farming system		Grazing		Sampling time x Farming system		Sampling time x Grazing		Farming system x Grazing		Sampling time x Farming system x Grazing	
			Pseudo-F	p	Pseudo-F	p	Pseudo-F	p	Pseudo-F	p	Pseudo-F	p	Pseudo-F	p	Pseudo-F	p
Structure Maintenance	AggSurf (Score)	Aggregate stability (0-2cm)	0,27	ns	14,63	**	0,06	ns	0,37	ns	0,00	ns	0,78	ns	1,20	ns
	AggSoil (Score)	Aggregate stability (2-10cm)	2,53	ns	6,71	*	0,65	ns	1,97	ns	0,52	ns	3,67	ns	1,32	ns
	Beerkan (ml/m)	Infiltration rate	3,66	ns	5,77	ns	2,27	ns	7,35	ns	6,29	ns	7,01	ns	5,92	ns
	VESS (Score)	Soil structure assessment	Neg.	N/A	1,41	N/A	0,51	N/A	Neg.	N/A	0,00	N/A	0,49	N/A	Neg.	N/A
Nutrient Cycling	AEMNO3 (um/cm^2/d)	NO_3^- fixed on ion exchange membranes	0,21	ns	1,22	ns	1,35	ns	1,04	ns	0,03	ns	0,61	0 ns	0,23	ns
	NO_3^- (mg/kg)	Available NO_3^-	1,42	ns	2,81	ns	2,38	ns	0,77	ns	1,92	ns	1,28	ns	1,53	ns
	NH_4^+ (mg/kg)	Available NH_4^+	7,65	ns	0,57	ns	0,49	ns	0,40	ns	0,82	ns	0,07	ns	0,80	ns
Carbon Transformation	POXC (mg/kg)	Active carbon (Permanganate oxidizable carbon)	1,96	ns	6,31	*	3,40	ns	0,34	ns	1,16	ns	1,23	ns	0,13	ns
	Lamina (%deg/d)	Lamina bait	152,07	***	0,38	ns	2,17	ns	0,32	ns	2,19	ns	0,20	ns	0,32	ns
	Situresp (Abs)	Basal soil respiration	43,35	***	5,27	*	1,58	ns	0,34	ns	0,02	ns	0,62	ns	0,18	ns

Note: VESS score for F-values are negative (Neg.), meaning unavailable (N/A) P values. If the values exhibit substantial similarity across groups, it will generally result in a negative sum of squares, leading to a negative mean square and, consequently, a negative F-value.

Table 4.2: Pairwise comparisons of the Biofunctool parameters which differed significantly in within study factors. Bold values: significant difference (P < 0.05).

	AggSurf		Lamina		Situresp	
	Pseudo-F	p	Pseudo-F	p	Pseudo-F	p
Farming system						
Conv. vs cons.	3,35	0,0154				
Conv. vs uncult.	5,02	**0,0021**				
Cons. vs uncult.	2,58	0,2990				
Sampling time	3,66	0,1614				
Season 1 vs season 2			12,33	**0,0001**	6,58	**0,0002**

4.1.2 *Nutrient cycling*

Linear models were employed to examine how farming systems and sampling time affected the Biofunctool® indicators (Tables 4.3 & 4.4). Nutrient cycling indicators (NO_3^-) exhibited noticeable responsiveness to the conservation agriculture system (P < 0.05) during both seasons, explaining more than 50% (R_2 > 50%) of the variance (Tables 4.3 & 4.4). The nutrient cycling parameters represented by AEMNO3, NO_3^-, and NH_4^+ showed that the conservational system had higher average values than the conventional and uncultivated system, following a different trend of structure maintenance (Table 4.3 & 4.4). Only in August 2022 was the NH_4^+ value higher for the uncultivated system (Avg. = 31.48 mg/kg) (Table 4.3). On average, all farming systems' NO_3^- and NH_4^+ values were relatively lower in the February 2023 season.

4.1.3 *Carbon transformation*

Tables 4.3 and 4.4 show that carbon transformation indicators only responded to the uncultivated system (P < 0.05) with SituResp for August 2022 and active carbon for February 2023. SituResp had a much higher mean (0.94 Abs) in August 2022 than in February 2023 (0.4 Abs). The farming system strongly influenced active carbon results, with the uncultivated systems having the highest average values (525 mg/kg) for both seasons. It is clear from Tables 4.3 & 4.4 that the Lamina results were much higher in February 2023, and the PERMANOVA table also indicated that sampling time strongly influenced the Lamina results (P < 0.0001) (Table 4.1).

4.2 Impact of farming systems and seasons on the soil health status using the Biofunctool® soil quality index.

A principal component analysis (PCA) was done on the Biofunctool® indicators to consolidate all the functional parameters and factors to study the relationships and patterns amongst the tested variables. A soil quality index was developed that combined the Biofunctool® indicator values into a single score based on the weightings derived from the PCA. For the winter season (August 2022), the first and second axes represented 36% and 21.7% of the total variability, respectively (Figure 4.1A). The variability of the first axis was mainly explained by the active carbon (20%), Beerkan (18%), and SituResp (14%). For this dimension, the variables were positively correlated with each other, except for VESS and Lamina, which were negatively correlated. The SQI (Figure 4.1B) differentiated between the uncultivated fields and the conventional agriculture system. The difference was mainly related to the structure maintenance linked to AggSurf having the highest contribution, followed by VESS and Beerkan. The SQI values for the uncultivated had a total score of 0.6, almost double that of the conventional system. The conservation agriculture system scored 0.51, closely related to the uncultivated fields (Figure 4.1B). The structural maintenance function was the most contributing function explaining the differences in the SQI.

In the summer season, February 2023, the first and second axes represented 41.2% and 36.3% of the total variability, respectively (Figure 4.2A). The primary components driving the first dimension were Lamina, AggSoil, AggSurf, and active carbon, contributing 21%, 20%, 19%, and 18%, respectively, to its composition. The SQI (Figure 4.2B) differentiated between the uncultivated and conventional systems. The distinction primarily correlated with two indicators associated with soil functions: carbon transformation (Lamina) and soil structure (AggSoil and AggSurf), both of which held greater significance compared to nutrient cycling. The SQI values for the uncultivated system scored 0.56, almost double that of the conventional system. The conservation agriculture system scored 0.54, closely related to the uncultivated system (Figure 4.2B). The structural maintenance function was the most contributing ecosystem function, explaining the differences in the SQI.

When the principal component analysis was done on the combined season data, the first and second axes represented 31.5% and 23.9% (Figure 4.3A), which were relatively lower when compared to the PCAs of the individual season data (Figures 4.1A & 4.2A). Active carbon (20%) and Beerkan (18%) were the main contributors towards the first dimension. The Lamina indicator results were the primary explanation for the second dimension in the PCA (33%).

The SQI delineated differences between the uncultivated and conventional systems, primarily associated with two indicators connected to soil functions: Lamina, active carbon (carbon transformation), and AggSoil, AggSurf (soil structure), having the highest contribution to the PCA dimensions. The SQI values for the uncultivated system of 0.49 were almost double that of the conventional system. The conservation agriculture system scored 0.44, closely related to the uncultivated system (Figure 4.3B). The structural maintenance function played the most significant role in explaining the differences in the SQI, closely followed by the carbon transformation function.

Table 4.3: Univariate statistics of the Biofunctool® indicators sampled in August 2022 across the sampling sites (n=33).

			Conventional					Conservation					Uncultivated				
Soil function	**Measured variable**	**Indicator**	Avg	Min	Max	P-Value	R^2	Avg	Min	Max	P-Value	R^2	Avg	Min	Max	P-Value	R^2
Soil Structure	Aggregate stability (0-2cm)	AggSurf (Score)	2.2	1.3	3.9	ns	73%	4.13	3.50	4.80	ns	58%	4.58	3.3	5.8	ns	33%
	Aggregate stability (2-10cm)	AggSoil (Score)	3.07	1.8	5.5	ns	50%	3.08	2.10	4.50	ns	26%	3.55	2.6	4.5	ns	50%
	Infiltration rate	Beerkan (ml/m)	18.8	6.9	40.3	ns	84%	49.28	13.4	112.4	ns	58%	117.35	14.3	341.7	ns	32%
	Soil structure assessment	VESS (Score)	1.67	1	2	ns	83%	2.13	1.50	3.00	ns	50%	2.5	1	3	ns	58%
Nutrient Cycling	NO_3^-fixed on ion exchange membranes	Ion exchange AEMNO3 (um/cm^2/d)	0.01	0.001	0.03	ns	16%	0.01	0.001	0.03	ns	50%	0.01	0.001	0.02	ns	35%
	Available NO_3^-	NO_3^- (mg/kg)	10.07	1.7	24.7	ns	30%	27.58	4.6	62.1	*	66%	20.35	4.20	48.80	ns	29%
	Available NH_4^+	NH_4^+ (mg/kg)	19.53	15.6	22.3	ns	83%	29.31	19.24	54.3	*	55%	31.48	21.10	43.60	ns	32%
Carbon transformation	Active carbon (Permanganate oxidizable carbon)	Active carbon (mg/kg)	281.6	409.7	153.5	ns	16%	444.98	393.1	468.5	ns	14%	578.1	359.10	825.10	ns	46%
	Lamina bait	Lamina (%deg/d)	0.01	0.01	0.02	ns	84%	0.01	0.001	0.02	ns	29%	0.03	0.01	0.07	ns	35%
	Basal soil respiration	Situresp (Abs)	0.69	0.5	0.8	ns	46%	0.67	0.63	0.71	ns	61%	0.94	0.72	1.09	*	59%

ns for $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 4.4: Univariate statistics of the Biofunctool® indicators sampled in February 2023 across the sampling sites n=33).

			Conventional		Conservational		Uncultivated	

Soil function	Measured variable	Indicator	Avg	Min	Max	P-Value	R^2	Avg	Min	Max	P-Value	R^2	Avg	Min	Max	P-Value	R^2
Soil Structure	Aggregate stability (0-2cm)	AggSurf (Score)	1.92	1.30	2.57	ns	85%	3.98	2.97	5.33	ns	27%	5.74	5.17	6.00	ns	27%
	Aggregate stability (2-10cm)	AggSoil (Score)	1.97	1.50	2.73	ns	85%	4.31	2.90	5.57	ns	42%	5.27	4.73	5.90	ns	29%
	Infiltration rate	Beerkan (ml/m)	37.37	15.17	75.10	ns	84%	67.31	13.17	140.67	ns	25%	39.76	14.30	81.53	ns	61%
	Soil structure assessment	VESS (Score)	1.67	1.00	2	ns	85%	2.13	1.50	3.00	ns	25%	2.5	1.00	3.00	ns	37%
Nutrient Cycling	NO_3^-fixed on ion exchange membranes	Ion exchange AEMNO3 (um/cm^2/d)	0.01	0.01	0.001	ns	85%	0.03	0.01	0.04	*	52%	0.01	0.001	0.02	ns	64%
	Available NO_3^-	NO_3^- (mg/kg)	4.16	1.18	5.84	ns	84%	26.39	4.02	56.95	*	54%	6.29	1.19	13.52	*	66%
	Available NH_4^+	NH_4^+ (mg/kg)	8.35	1.87	21.29	ns	44%	12.9	0.53	44.26	ns	24%	4.47	0.76	7.49	ns	17%
Carbon transformation	Active carbon (Permanganate oxidizable carbon)	Active carbon (mg/kg)	271.08	216.39	314.46	ns	15%	372.92	345.97	394.03	ns	30%	471.9	276.24	667.27	*	49%
	Lamina bait	Lamina (%deg/d)	0.03	0.03	0.04	ns	26%	0.04	0.04	0.05	ns	24%	0.05	0.04	0.06	ns	32%
	Basal soil respiration	Situresp (Abs)	0.27	0.26	0.29	ns	30%	0.27	0.15	0.41	ns	21%	0.4	0.14	0.56	ns	40%

ns for $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

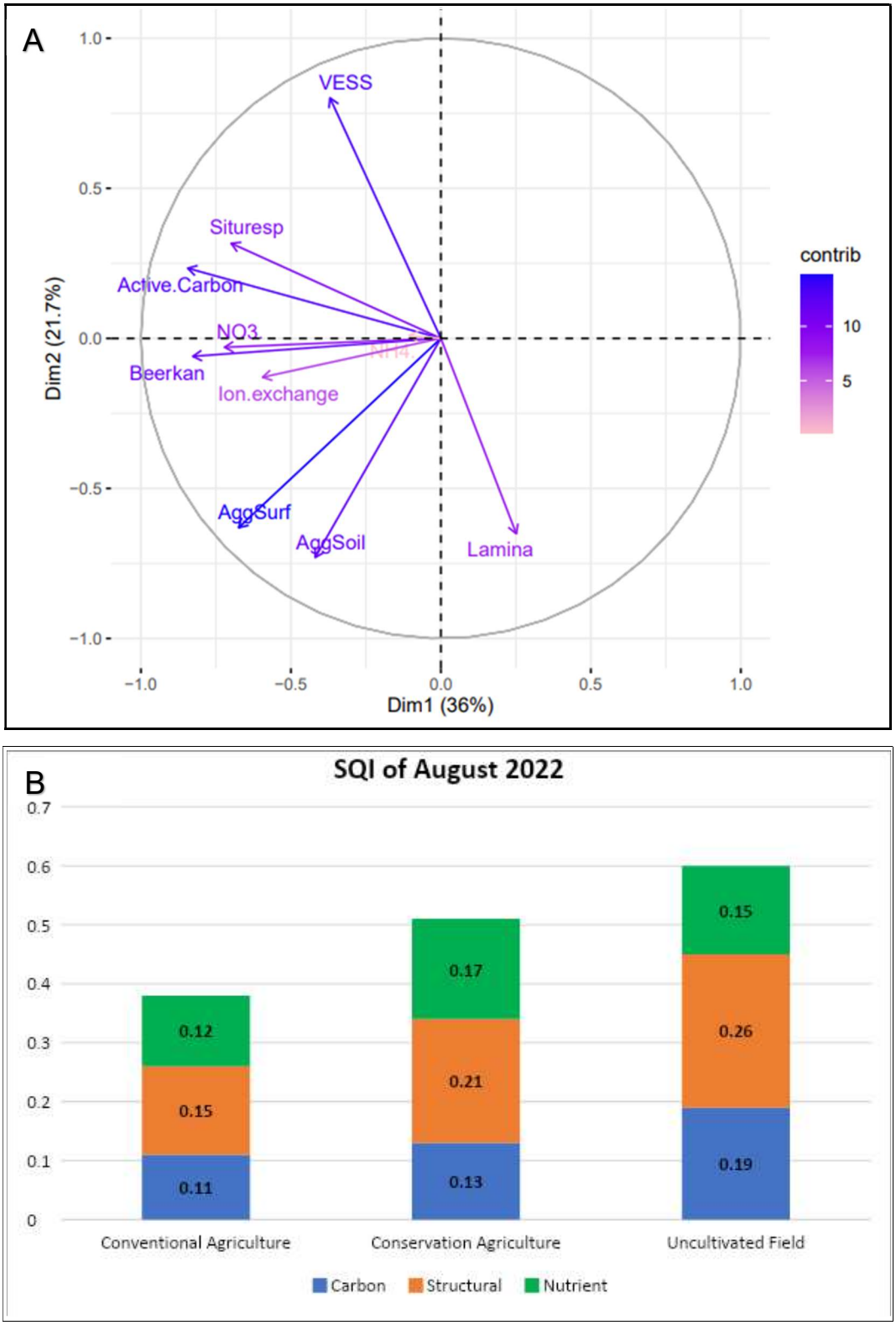


Figure 4.1: A) Principal component analysis performed on the Biofunctool® indicators measured in August 2022. **B)** Integration of the Biofunctool® parameters into the soil quality index (SQI) for each farming system in August

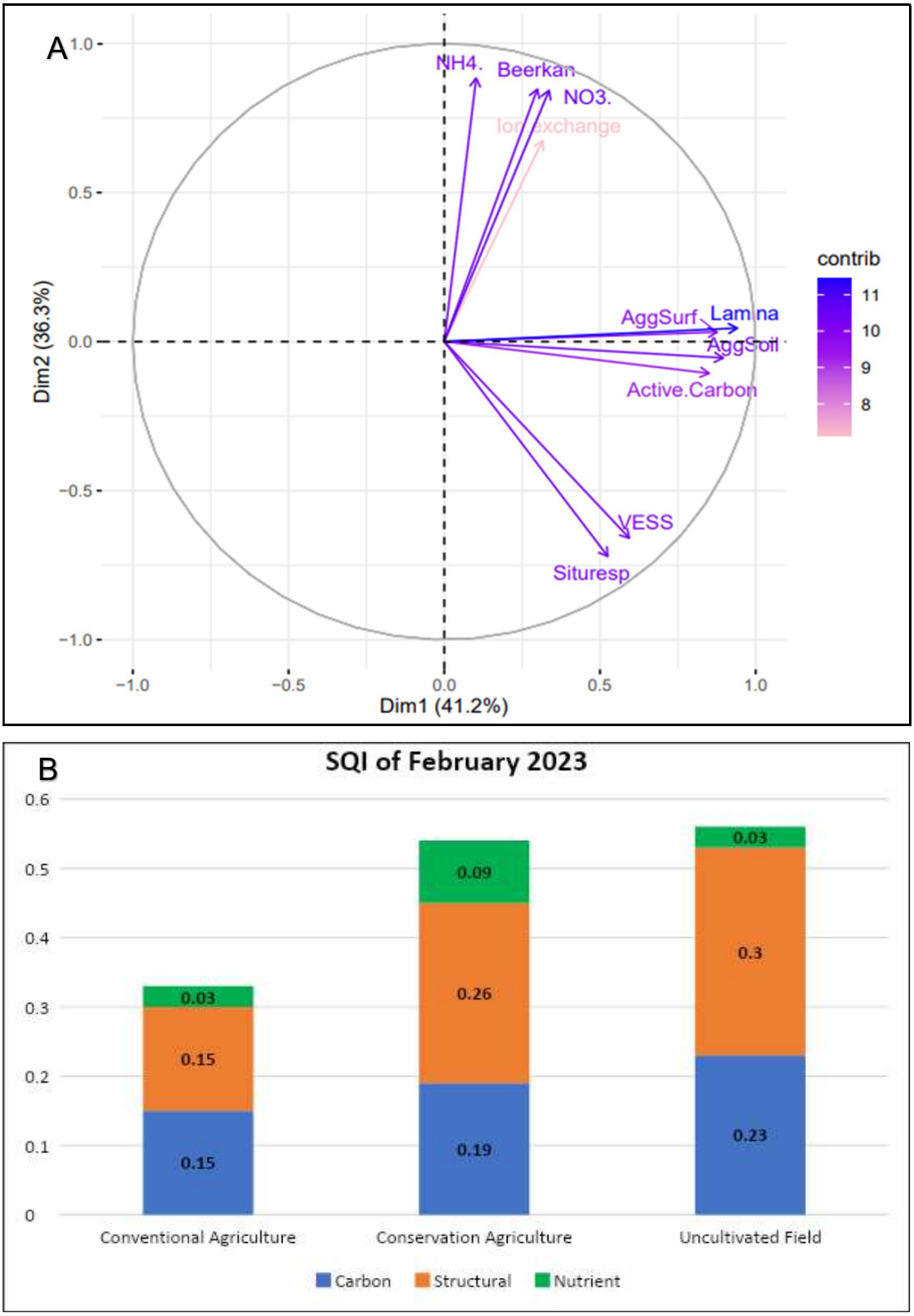


Figure 4.2: A) Principal component analysis performed on the Biofunctool® indicators for all 11 sampling points in February 2023. **B)** Soil quality index performed for each farming system in February 2023.

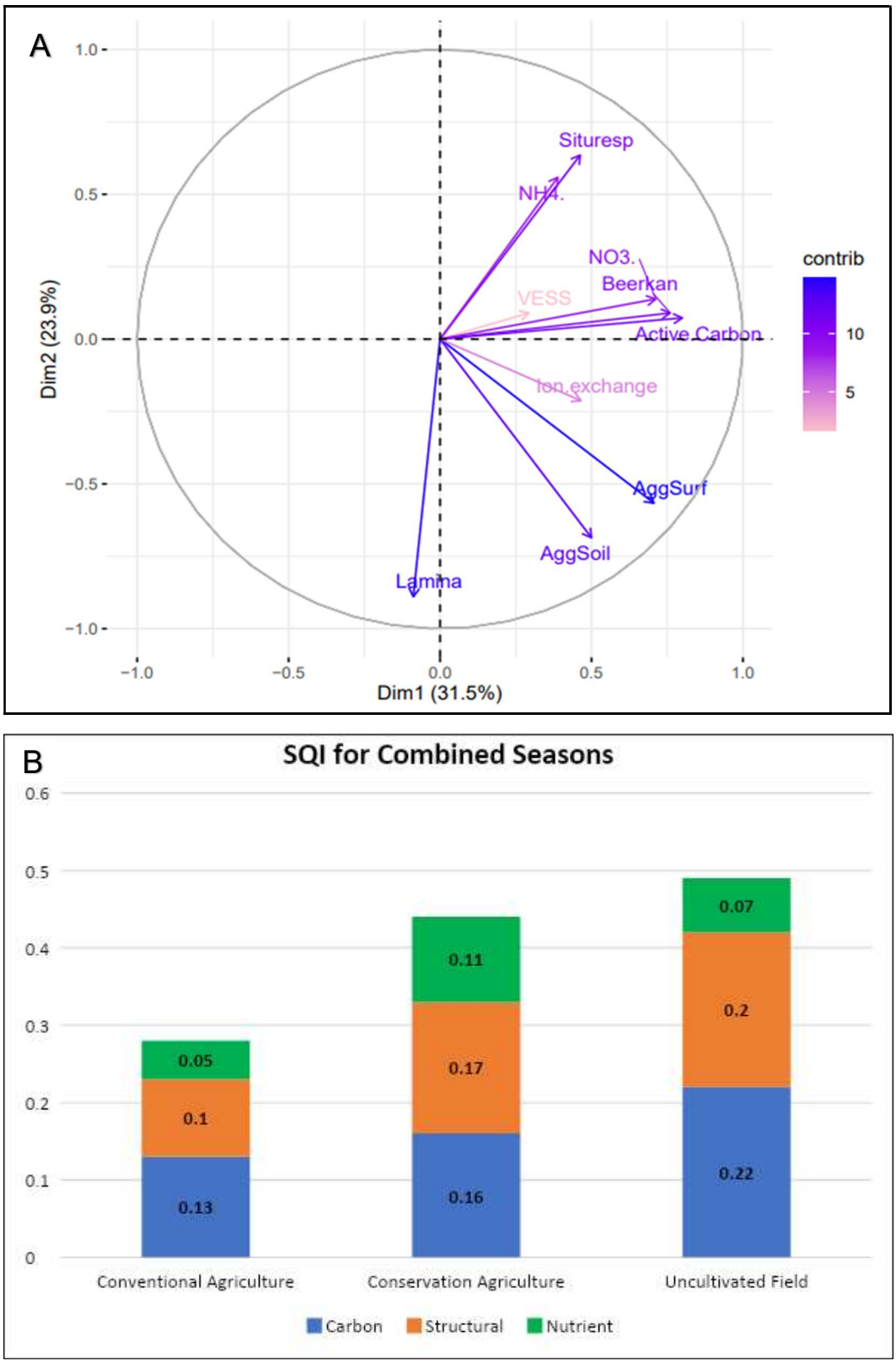


Figure 4.3: A) Principal component analysis performed on the Biofunctool® indicators for 22 sampling points across the two seasons. **B)** Soil quality index performed for each farming system across the two seasons

4.3 The influence of the grazing, seasons, and farming system on selected biological indicators

A PERMANOVA analysis was performed on the additional biological parameters (active carbon, SOC, FDA and β-glucosidase), which showed a significant effect (P<0.0001) on farming systems and sampling season (Table 4.5). Furthermore, significant interactions between sampling time x farming systems and farming systems × grazing were present. The three-way interaction was not significant (P>0.05). The farming systems exhibited distinct variations between grazed and non-grazed treatments (Figure 4.4, A-D) and displayed divergences in the sampling times (Figure 4.5, A-D). Notably, in August 2022, the highest levels of variability and values for all soil variables were observed (Figure 4.5, A-D).

Table 4.5: PERMANOVA results of measured soil indicators across the four seasons and sampling sites.

Factor	df	SS	MS	Pseudo-F	P (perm)
Sampling time	3	176,76	58,92	41,581	**0,0001**
Farming system	2	115,49	57,743	40,751	**0,0001**
Grazing	1	2,0403	2,0403	1,440	0,2136
Sampling time x Farming system	6	25,089	4,1815	2,951	**0,0006**
Sampling time x Grazing	3	1,953	0,651	0,459	0,8705
Farming system x Grazing	2	14,131	7,0655	4,986	**0,0010**
Sampling time x Farming system x Grazing	6	8,476	1,4127	0,997	0,4418
Res	112	158,7	1,417		
Total	135	540			

Bold values: significant variables (P < 0.05)

4.3.1 *Active carbon (POXC)*

The conventional and conservation systems had similar active carbon averages in the non-grazed system. Overall, the average values of active carbon for conservation agriculture (319.59 mg/kg) and uncultivated systems (478.2 mg/kg) tended to be higher in grazed conditions (Figure 4.4A). However, the conventional system exhibited higher average values in a non-grazed system (246.62 mg/kg), deviating from this trend (Figure 4.4A). The trend in active carbon values exhibited consistency, with the conventional system consistently showing the lowest values, whether in grazed or non-grazed conditions. In contrast, the uncultivated system consistently displayed the highest values, regardless of grazing. August 2022 had the most variability and highest values overall for all farming systems (Figure 4.5A). August 2021 had the lowest averages for the conventional system (131.24 mg/kg) and the uncultivated system (216.67 mg/kg) compared to the other seasons. The conservation system had the

lowest average (207.07) in March 2022. When variables were evaluated individually, active carbon was the only one to present an increase through time for the conventional system.

4.3.2 *Fluorescein diacetate hydrolysis (FDA)*

The average FDA values for the conventional system (grazed = 105 mg p-nitrophenol kg^{-1} h^{-1} and non-grazed = 107 mg p-nitrophenol kg^{-1} h^{-1}) and the conservation agriculture system (grazed = 109 mg p-nitrophenol kg^{-1} h^{-1} and non-grazed = 110 mg p-nitrophenol kg^{-1} h^{-1}) demonstrate minimal variation between the grazed and non-grazed conditions (Figure 4.4B). Only the uncultivated system had higher average values in grazed conditions (122 mg p-nitrophenol kg^{-1} h^{-1}). Fluorescein diacetate hydrolysis exhibited its lowest values in August 2021 (Figure 4.5B) across all farming systems. These values ranged from 98 mg p-nitrophenol kg^{-1} h^{-1} for the conservation system to 104 mg p-nitrophenol kg^{-1} h^{-1} for the uncultivated system. Conversely, the highest values for all farming systems were recorded in August 2022, with an average value of 199 mg p-nitrophenol kg^{-1} h^{-1} for the conservation agriculture system to 356 mg p-nitrophenol kg^{-1} h^{-1} for the uncultivated system. Notably, during August 2021 and August 2022, the conservation agriculture (77.44 mg p-nitrophenol kg^{-1} and 75.3 mg p-nitrophenol kg^{-1}) system exhibited lower values than the conventional system. In contrast, the conservation system consistently displayed higher values across all other seasons and soil variables than the conventional system.

4.3.3 *β-glucosidase*

The average values of β-glucosidase activity were notably elevated under grazed conditions for the conservation system (6259 mg kg^{-1} h^{-1}) and the uncultivated system (6754 mg kg^{-1} h^{-1}). In contrast, the conventional system exhibited higher average values under non-grazed conditions, with an average value of 3490 mg kg^{-1} h^{-1}, surpassing the values observed in the grazed system (2795 mg kg^{-1} h^{-1}) (Figure 4.4C). The peak levels of β-glucosidase activity were observed across all farming systems during the August 2022 season. These levels ranged from 4272 mg kg^{-1} h^{-1} for the conventional system to 11729 mg kg^{-1} h^{-1} for the uncultivated system, as depicted in Figure 4.5C. Conversely, the lowest average values were recorded during different periods: for the conventional system in March 2022 (3329 mg kg^{-1} h^{-1}), for the conservation system in August 2021 (5314 mg kg^{-1} h^{-1}), and for the uncultivated system in February 2023 (5828 mg kg^{-1} h^{-1}).

4.3.4 *Soil organic carbon*

In the case of the conventional system, the non-grazed fields yielded higher average values of soil organic carbon (SOC), specifically at 0.9%, as illustrated in Figure 4.4D. In contrast, the conservation system exhibited minimal differences between the grazed (0.9%) and non-grazed conditions (0.8%). Conversely, the uncultivated system displayed significantly higher SOC levels in the non-grazed condition, reaching a percentage of 1.34%. Across all farming systems, the most modest readings, ranging from 0.51% to 0.75% for conventional to uncultivated systems, were detected during the March 2022 season. The second lowest figures were observed in February 2023. Conversely, the peak values, spanning from 1.1% for the conventional and conservation system to 1.95% for the uncultivated system, were recorded in August 2022 across all seasons, as depicted in Figure 4.5D.

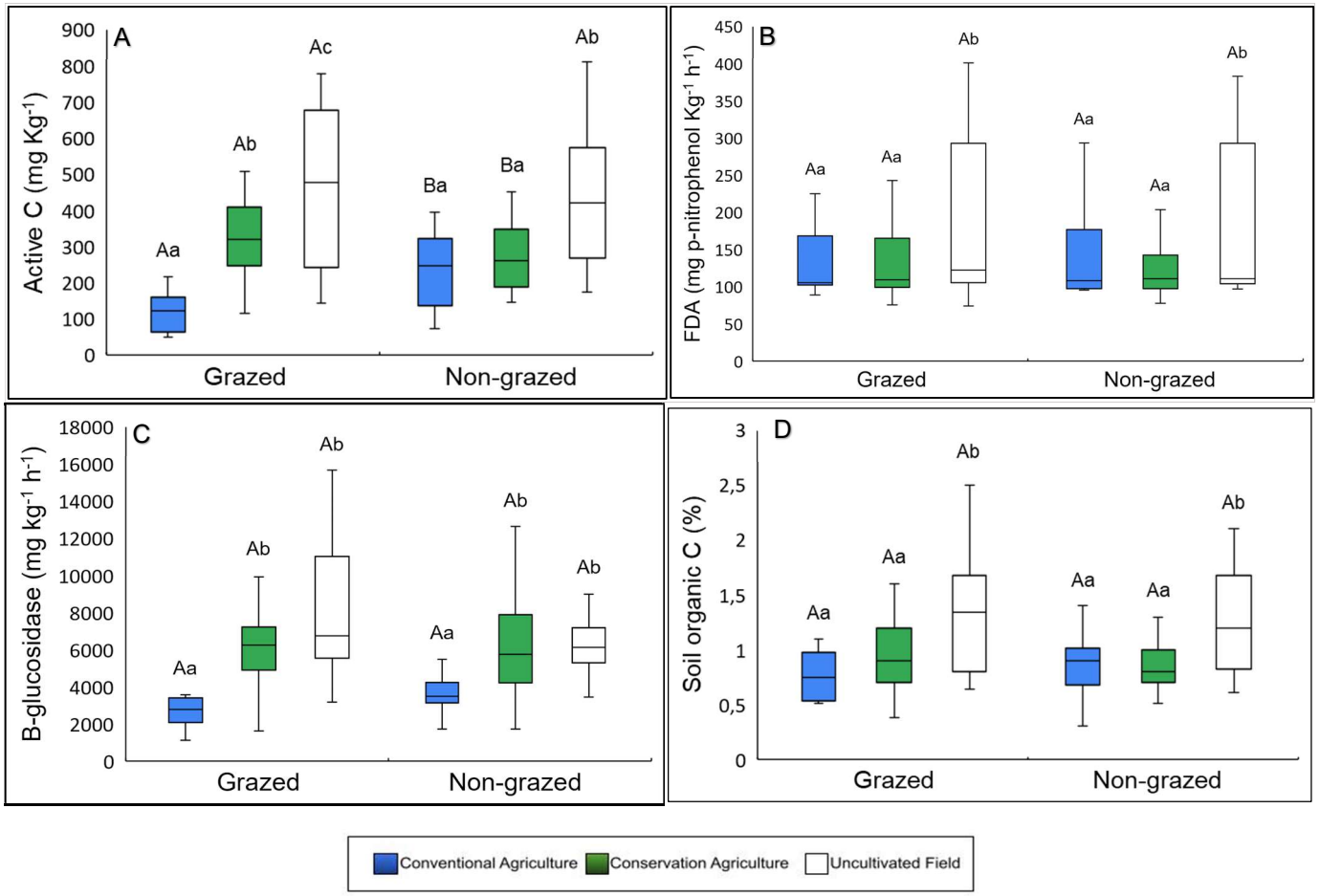


Figure 4.4: The Box and Whisker plots comparing the influence of farming system and Grazing on **A)** active carbon, **B)** FDA, **C)** B-glucosidase and **D)** soil organic carbon". Different capital letters: significant difference among the same farming system in different grazing treatments ($p < 5$ %). Different small letters: significant differences among different farming systems in the same grazing treatment

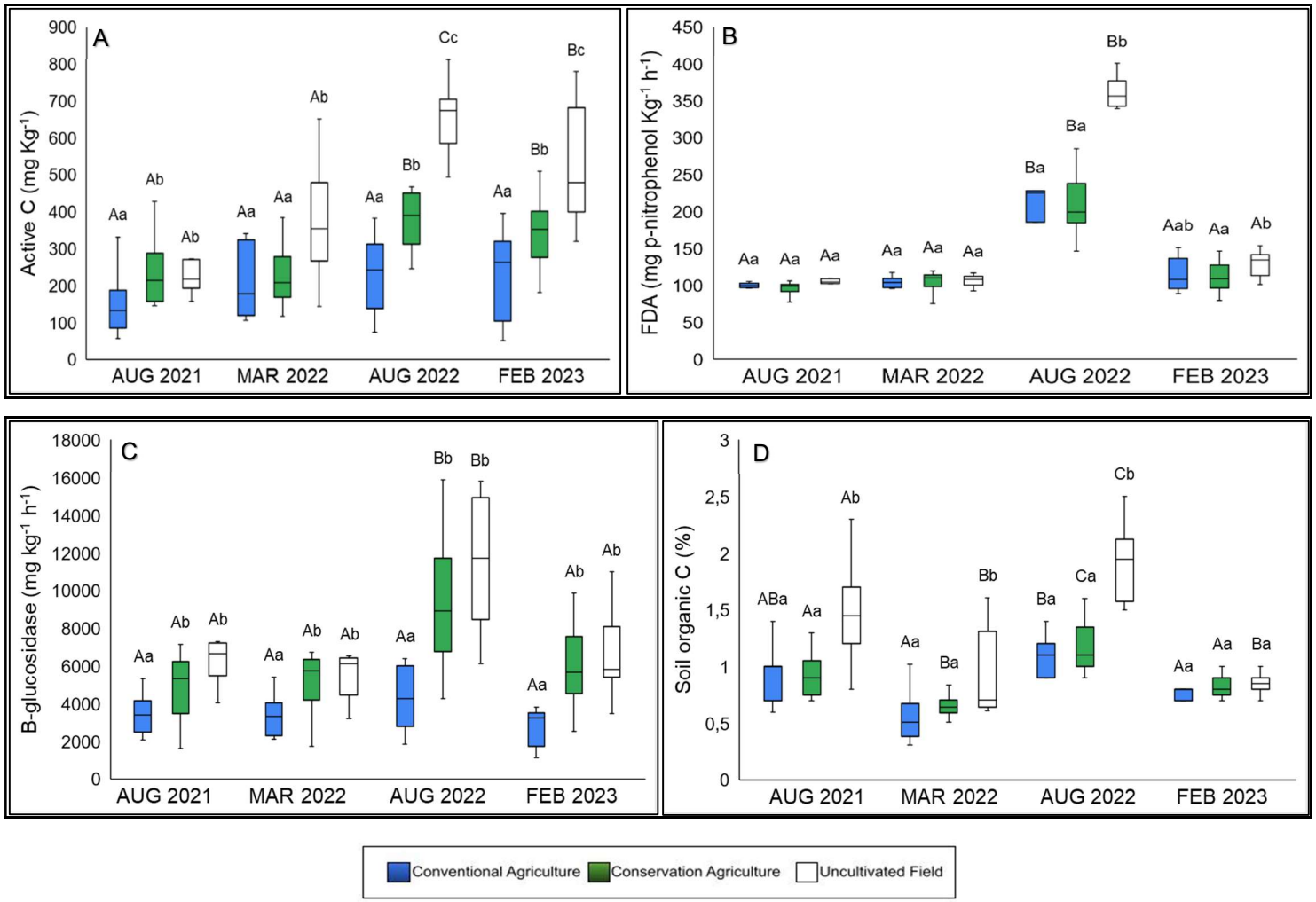


Figure 4.5: The Box and Whisker plots compare the influence of farming system and time of sampling on A) active carbon, B) FDA, C) B-glucosidase, and **D)** soil organic carbon. Different capital letters: significant difference among the same farming system in different sampling times ($p < 5$ %). Different small letters: significant difference among different farming systems in the same sampling time.

4.4 Incorporating enzymes and soil organic carbon into the soil health assessment.

Principal component analysis was also performed on the Biofunctool® indicators with enzyme activity and soil organic carbon for the August 2022 and February 2023 season. These two dimensions explained more than 50% of the dataset variability (Figure 4.6). The first dimension represented 34% of the variability and was mainly related to the FDA, SituResp, and SOC, which contributed to 18%, 17%, and 16% of the first dimension, respectively. The second dimension of the PCA accounted for 21.8% of the variability and was mainly explained by AggSurf (24%) and AggSoil (23%) indicator results. Table 4.6 illustrates that FDA was only strongly correlated to the Biofunctool® indicators: SituResp, SOC and NH_4^+, which can be seen in the PCA (Figure 4.6).

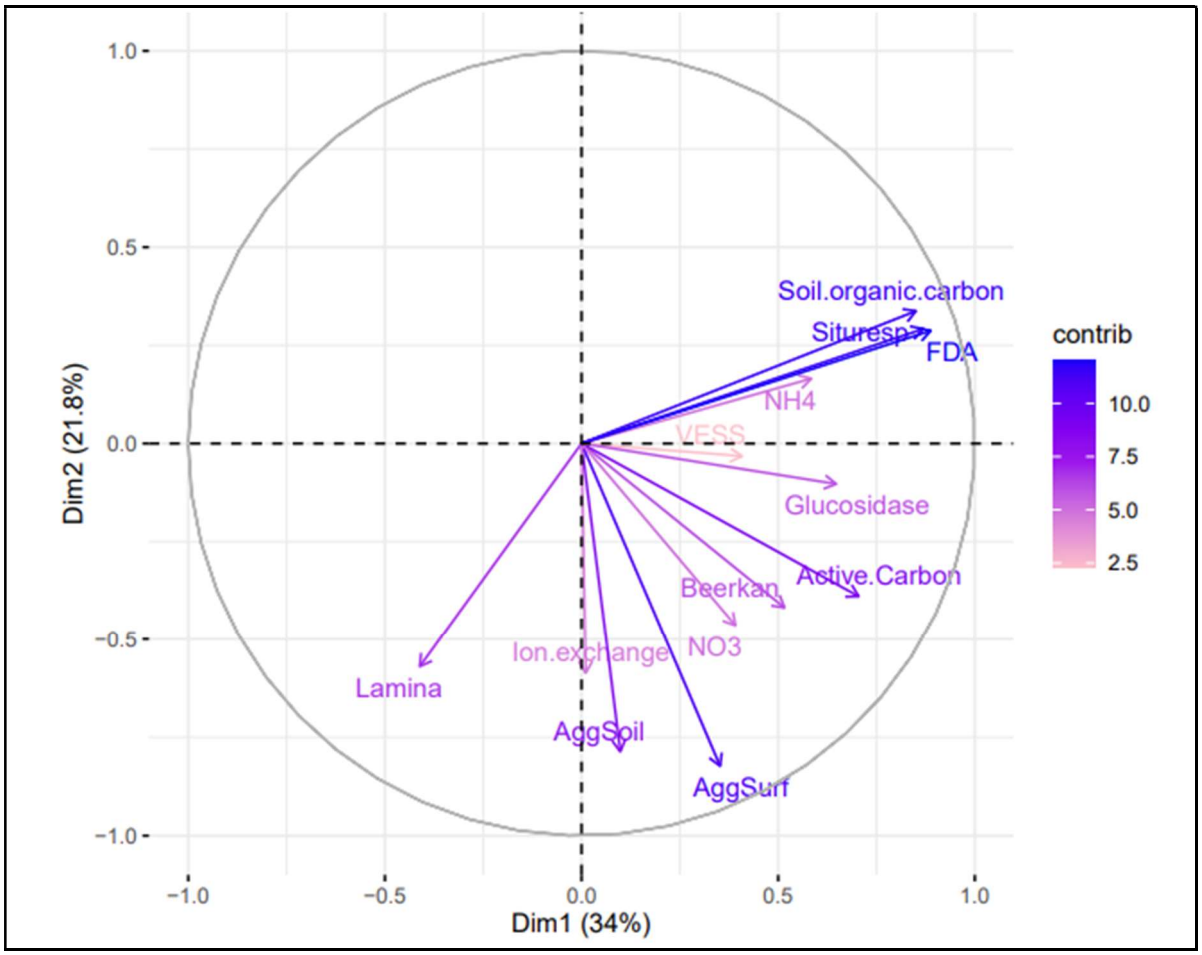


Figure 4.6: Principal component analysis performed on the Biofunctool® indicators, enzymes, and SOC for 33 sampling points in August 2022 and February 2023 season.

Table 4.6: Pearson correlation matrix per soil function across the Ngaka Modiri Molema and Thabo Mofutsanyana district for the August 2022 and February 2023 seasons. (values indicated in bold are significantly correlated).

	AggSurf	AggSoil	Beerkan	VESS	Active carbon	Lamina	Situresp	Ion.exchange	NO_3^-	NH_4^+	FDA	Glucosidase	SOC
AggSurf (Score)	1.00	0.80	0.38	0.12	0.53	0.44	0.07	0.25	0.33	0.06	0.14	0.44	0.06
AggSoil (Score)	0.80	1.00	0.25	0.11	0.32	0.47	-0.07	0.18	0.09	-0.13	-0.10	0.20	-0.12
Beerkan (ml/m)	0.38	0.25	1.00	0.11	0.55	-0.15	0.24	0.29	0.55	0.30	0.33	-0.02	0.27
VESS (Score)	0.12	0.11	0.11	1.00	0.49	-0.06	0.39	-0.16	-0.04	-0.19	0.22	0.34	0.32
Active carbon (mg/kg)	0.53	0.32	0.55	0.49	1.00	-0.09	0.55	0.14	0.33	0.14	0.42	0.37	0.42
Lamina (%deg/d)	0.44	0.47	-0.15	-0.06	-0.09	1.00	-0.55	0.08	-0.20	-0.44	-0.36	0.00	-0.36
Situresp (Abs)	0.07	-0.07	0.24	0.39	0.55	-0.55	1.00	-0.16	0.15	0.42	0.84	0.50	0.79
Ion. exchange ($um/cm^2/d$)	0.25	0.18	0.29	-0.16	0.14	0.08	-0.16	1.00	0.70	0.05	-0.22	-0.01	-0.22
NO_3^- (mg/kg)	0.33	0.09	0.55	-0.04	0.33	-0.20	0.15	0.70	1.00	0.44	0.14	0.15	0.02
NH_4^+ (mg/kg)	0.06	-0.13	0.30	-0.19	0.14	-0.44	0.42	0.05	0.44	1.00	0.58	0.28	0.47
FDA (mg p-nitrophenol kg^{-1} h^{-1})	0.14	-0.10	0.33	0.22	0.42	-0.36	**0.84**	-0.22	0.14	0.58	1.00	0.56	**0.95**
Glucosidase (mg kg^{-1} h^{-1})	0.44	0.20	-0.02	0.34	0.37	0.00	0.50	-0.01	0.15	0.28	0.56	1.00	0.57
Soil.organic.carbon (%)	0.06	-0.12	0.27	0.32	0.42	-0.36	**0.79**	-0.22	0.02	0.47	0.95	0.57	1.00

Chapter 5: Discussion

5.1 Introduction

This study evaluated soil health in conservation agriculture using a multi-functional approach. The study considered the correlations of various soil components and employed a standardised set of preselected soil indicators. By integrating these indicators into a multivariate analysis, valuable insights were gained on how different agricultural practices within a semi-arid environment affect soil functions. The study encompassed various farming systems, including conventional agriculture, conservation agriculture systems, and uncultivated fields, which served as a reference baseline for comparison. Different Biofunctool® indicators were employed to showcase variations in soil health across these farming systems. The study also made use of active carbon in the form of permanganate oxidizable carbon (POXC), soil organic carbon (SOC) and the activities of B-glucosidase and fluorescein diacetate (FDA) to evaluate the effects of different farming systems located in two different provinces of South Africa.

5.2 Biofunctool® index as a measure of soil ecosystem functions

5.2.1 *The impact of different farming systems and seasons on the structure maintenance function*

The study observed a clear positive impact of conservation agriculture on soil structure maintenance parameters: aggregate stability and VESS (Figure 4.1 & 4.2), consistent with many other studies (Pheap *et al.,* 2019; Sithole *et al.,* 2019; Swanepoel *et al.,* 2018). The improved aggregate stability observed under conservation agriculture aligns with findings from several studies, including those by Kulagowski *et al.* (2021) and Gura *et al.* (2022), conducted in similar agro-ecological conditions. Improved aggregate stability can be explained by the decomposition of plant residues, especially root systems (Sithole *et al.*, 2019) and the activity of soil fauna (Fiorini *et al.,* 2020). By minimising soil disturbance through reduced tillage and enhancing residue cover, organic matter in the soil can act as a bonding agent for mineral particles (Brady & Weil, 2014), leading to an improvement in the stability of soil aggregates (Sithole *et al.*, 2019).

An increase in water infiltration was evident within the conservation system and aligns with findings by Fu *et al.* (2021), Pheap *et al.* (2019) and Ranaivoson *et al.* (2017). All the studies have consistently shown that water infiltration tends to increase in soils covered by residues

and in no-tillage agricultural practices. Residue cover plays a crucial role in intercepting a portion of rainfall, mitigating soil crusting and reducing soil losses via runoff (Thierfelder *et al.,* 2015). Consequently, this contributes to an augmented rate of soil water infiltration. These improvements in infiltration are often associated with the enhanced stability of aggregates near the soil surface, particularly in areas where soil organic carbon (SOC) improvements are most pronounced (Ranaivoson *et al.,* 2017). Furthermore, the greater number and continuous presence of macropores, which facilitate the rapid movement of water into the soil profile, are made available due to the absence of tillage. These factors collectively contribute to increased water infiltration rates, a phenomenon frequently linked to the absence of tillage and improved soil management practices (Page, 2020). In February 2023, lower infiltration rates were observed across all farming systems, with the uncultivated fields experiencing the most significant decrease. This can be attributed to the substantial rainfall during the sampling period. Many of the sampling sites displayed signs of flooding, which had a dampening effect on infiltration rates.

5.2.2 *The impact of different farming systems and seasons on the nutrient cycling function*

The higher soil nitrogen levels (NO_3^- and NH_4^+) in the CA systems can be attributed to the decomposition of crop residues, which play a fundamental role in the nutrient cycle (Fu *et al.,* 2021; Kodzwa *et al.,* 2020; Page *et al.,* 2020). Returning crop residues to the soil serves as a means to increase the concentrations of organic carbon, nitrogen, potassium, and available phosphorus in the soil. Additionally, incorporating crop residues into the soil protects against nutrient loss while improving the accessibility of essential nutrients (Fu *et al.,* 2019; Pheap *et al.,* 2019).

The elevated nitrogen levels observed in the CA system, in contrast to the uncultivated field and conventional system, can be ascribed to the inclusion of legume crops (Shah *et al.,* 2021). The use of legumes, such as soybean (*Glycine max*), in crop rotations contributes residual nitrogen to agricultural systems. In this study, soybean is a common rotational crop amongst the sampled sites, leading to increased nitrogen levels due to the nitrogen-fixing capacity of legumes during their growth (Thierfelder *et al.,* 2013).

The variation in nitrogen values can also be attributed to seasonal effects, as noted by Madegwa & Uchida (2021) and Sherbine *et al.* (2023). In our study, the February 2023 sampling was the summer season, characterised by increased rainfall. During this period, soils exhibit higher moisture levels and temperatures, which stimulate microbial activity compared

to drier, colder seasons (Mettauer *et al.,* 2022; Madegwa & Uchida, 2021). Interestingly, we found higher nitrogen rates in August 2022 than in February 2023, which does not readily account for the increased values. The lower nitrogen values for February 2023 could be explained by the high rainfall and flooding of soils at the time of sampling. Studies have indicated that saturated soils can cause denitrification and leaching of N (Cregger *et al.,* 2014; Schimel, 2018). Soils for February 2023 were waterlogged, which was clearly evident with the lower infiltration rates (Table 4.3). Waterlogged soils cause anaerobic conditions, where certain bacteria can convert nitrates (NO_3^-) in the soil into gaseous nitrogen compounds, such as nitrous oxide (N_2O) and nitrogen gas (N_2), in a process called denitrification. This reduces nitrogen availability for plants, potentially leading to low nitrogen levels in the soil (Schimel, 2018). Excessive soil moisture can cause nitrogen to leach out of the root zone and move deeper into the soil profile or groundwater. Leaching can result in the loss of nitrogen from the topsoil, making it less accessible to plant roots. This can lead to nitrogen deficiency in the upper soil layers (Cregger *et al.,* 2014).

Another factor to consider is the timing of harvesting, as discussed by Pheap *et al.* (2021). Residues present on top of each farming system were in different stages of decomposition and mineralization at the time of sampling. For instance, harvesting had already occurred in the conservation fields at the time of sampling in August 2022, initiating the decomposition process, and new cover crops were sown. Some conventional fields had already been harvested and were bare, while others had not been harvested yet at the time of sampling. The application of N fertilisers could also have an impact on the nitrogen results. After harvesting, some of the unused fertilisers could remain in the soil, increasing nitrogen levels (Kodzwa *et al.,* 2020). A study done by Madegwa & Uchida (2021) found that soil microbial activity was much higher in a cropland than a grassland. The higher values of NO^{3-} and NH_4^+ in the conventional system could be explained by the effect of tillage, which can mix the microbial habitat and unify the microbial community structure (Madegwa & Uchida, 2021).

Soil nitrate dynamics were evaluated using the Ion exchange membranes (AEMNO3). In our study, the AEMNO3 were not statistically significant in the multivariate and univariate analysis as the values were overall relatively low for all farming systems and seasons compared to studies done by Mettauer *et al.* (2022) and Thoumazeau *et al.* (2019). The low results can be attributed, in part, to variations in ion supply rates influenced by soil moisture levels in the field. The study sites are located in a semi-arid region (Nortje & Laker, 2021), with soils generally drier than other studies done with ion exchange membranes (Thoumazeau *et al.,* 2019). The diffusion path becomes lengthier and more convoluted when the soil becomes drier as the

larger pores cease to be saturated with soil solution. This limitation on diffusive flux mirrors the challenge faced by plant roots in natural field conditions as the soil undergoes drying (Qian & Schoenau, 2002). The elevated results observed in the ion exchange membranes in February 2023 could be attributed to the soil's waterlogged condition during the sampling period. This is likely because the membranes were left in the field for two weeks, during which the soils gradually dried up. Further investigation is needed to comprehend the use of ion exchange membranes in semi-arid climates, integrating measurements of soil moisture.

5.2.3 *The impact of different farming systems and seasons on the carbon transformation function*

In their study, Culman *et al.* (2012) suggested that POXC are well suited as a sensitive indicator of different management practices. Our results found that POXC were significantly higher in the conservation and uncultivated fields. These results are quite common, as confirmed by many studies (Koun *et al.,* 2023; Pheap *et al.,* 2019; Hok *et al.*, 2018). As per Hok *et al.* (2018), the elevated POXC findings may be linked to the consistent influx of biomass-C. This influx could lead to an upsurge in the labile SOC pools, attributed to increased root input, subsequently stimulating microbial activity. Conversely, continual soil tilling diminishes the provision of carbohydrates for microorganisms and soil enzyme activity, ultimately reducing microbial biomass-C (Hok *et al.,* 2018).

Measuring microbial activity within an ecosystem involves using soil basal respiration (BAS) and observing the organic carbon cycle. Among these indicators, basal respiration is particularly crucial in monitoring decomposition processes (Babur, 2019; Cheng & Xia, 2012). Our study revealed significantly higher SituResp values in the uncultivated fields. In contrast, no notable difference in SituResp values was observed between the fields under CA and those under conventional farming practices. The higher values of SituResp in the uncultivated fields can be explained by the presence of grass species (Thapa *et al.,* 2023) and an increase in manure in the fields from livestock (Adesoyin, 2020; Obriot *et al.,* 2016). The uncultivated fields are subject to livestock grazing and exclusively feature natural grass species. According to Thapa *et al*. (2023), grasses with densely packed fibrous root systems tend to generate more significant biomass with elevated C:N ratios, whereas legumes generally offer higher-quality residue characterised by lower C:N ratios. In contrast to legumes, grasses have greater C:N ratios, which may slow the breakdown of residues, leading to increased soil organic carbon (SOC) levels. Additionally, the extensive grass canopy covering and strong, thick root systems improve SOC stability and slow the pace of residue decomposition (Thapa *et al.,* 2023). Research conducted by Adesoyin (2020) illustrated how introducing carbon and

various nutrients through manure can lead to an elevation in microbial biomass and soil respiration rates, primarily attributed to the growth of bacterial populations.

Koun *et al.* (2023) observed in their study that labile carbon fractions and soil respiration were high in the early implementation of conservation agriculture. Although our studies did indicate an increase of POXC for conservation agriculture, CA fields were implemented for more than five years, and an equilibrium between short-term SOC mineralization and stabilisation could have been reached in these systems (Thapa *et al.,* 2023; Pheap *et al.,* 2019).

In the bait-lamina tests, we observed that the average feeding activity in August 2022 was relatively low. In contrast, during February 2023, the average feeding activity was higher in both the conservation agriculture (CA) system and the uncultivated field. A study conducted by Gavin-Centol *et al*. (2023) suggested that bait consumption can be influenced by factors such as drought and the timing of sampling. In our case, August 2022 experienced notably drier conditions compared to February 2023, and our sampling coincided with rainy conditions, potentially contributing to the increased feeding activity. It is worth noting that detritivore feeding appears to be positively affected by soil moisture levels (Mcinga *et al.,* 2020). Additionally, the application of NPK fertilisers could be another factor influencing the elevated feeding rates by detritivores. Research by Gavin-Centol *et al*. (2023) & Siebert *et al*. (2019) has demonstrated that fertilisers tend to reduce feeding activity. However, it is important to highlight that further investigation is needed to better understand the specific effects of fertiliser application rates and rainfall data on detritivore behaviour.

5.3 The influence of farming systems and seasons on active carbon, soil enzymes and soil organic carbon is as follows:

5.3.1 *Active carbon (POXC)*

Active carbon levels were investigated across four seasons in various farming systems, where some sites were subjected to grazing while others remained non-grazed. The elevated rates of POXC under the conservation system can be attributed to two primary factors. The first is the implementation of no-till management practices, which minimise soil disturbance (van Antwerpen *et al.,* 2021; Pheap *et al.,* 2019; Zuber *et al.,* 2017). The second is the utilisation of cover crops, which continuously contribute organic compounds through the decomposition of aboveground biomass and root exudates (Fu *et al.,* 2021; Kodzwa *et al.,* 2020; Patra *et al.,* 2019). The incorporation of biomass-derived carbon from the cover crops, encompassing both aboveground components and contributions from the root systems, coupled with the use of

no-tillage methods, can lead to the enhancement of labile carbon fractions, as noted by Hok *et al*. (2018). Additionally, these practices can also influence soil microbial communities (Koun *et al.,* 2023)

Research has shown that livestock integration can increase soil organic carbon and nitrogen values (Gallindo *et al.,* 2020; Bieluczyk *et al.,* 2017; Sanderson *et al.,* 2013). The conservation and uncultivated systems had much higher POXC values than the grazed systems. Elevated values can be linked to larger bacterial populations, which boost the soil microbial biomass closely associated with the soil's organic matter pool (Adesoyin, 2020). Soil microbial biomass significantly influences soil dynamics, acting as both a provider and receiver of plant-accessible nutrients and aiding in the conversion of these nutrients within the soil. As it decomposes, the microbial biomass consumes intricate organic substances to generate energy and biomass carbon, releasing surplus inorganic nutrients into the soil (Medegwa & Uchida, 2021; Adesoyin, 2020).

5.3.2 *Fluorescein diacetate hydrolysis (FDA)*

Several hydrolases, including proteases, lipases, and esterases, are responsible for catalysing the hydrolysis of fluorescein diacetate into fluorescein. These enzymes also contribute to the decomposition of organic matter, releasing nutrients accessible to plants (Green *et al.,* 2006; Shaw *et al.,* 2003). Therefore, the FDA can indicate the soil’s overall biological activity (Bera *et al.,* 2017).

Results of FDA were generally higher for the conservation systems than the conventional system, and these results are quite common and have been documented by many studies (Pittarello *et al.,* 2022; Galindo *et al.,* 2020; Muzangwa *et al.,* 2020; Bera *et al.,* 2017). The increased FDA activity can be attributed to elevated microbial activity, which is a consequence of the higher SOC content (Bera *et al.,* 2017; Rao *et al.,* 2017). This increase in SOC can be elucidated by crop rotations and residue retention in conservation agriculture (Muzangwa *et al.,* 2020), leading to greater biomass that supplies the energy needed for enhanced microbial activity (Lehman *et al.,* 2015). A brief episode of light rain occurred just before the sampling period, potentially accounting for the elevated results observed in August 2022. As described in the studies conducted by Rao *et al.* (2017) and Schimel (2018), soil rewetting due to light rain can enhance the accessibility of organic matter. This enhancement is brought about by the disruption of soil aggregates and cell lysis, which can trigger a surge in microbial turnover, consequently leading to a temporary upswing in extracellular enzyme activities.

Notably, FDA values were higher in the conventional farming system during August 2022, which may be attributed to soil disturbance caused by tilling. During the tilling process, crop residues are mixed into a larger volume of soil, accelerating the decomposition of organic matter. This increased contact between microorganisms and crop residues leads to a spike in FDA enzyme activity (Bera, 2017). It is also well-documented that soil enzymes are affected by litter quality (Bera, 2017; Rao et al., 2017). Rao *et al.* (2017) observed increased earthworm populations in a maize cropping system. Considering that most of our conventional sites were maize (Zea mays) fields, the maize harvesting may have temporarily boosted the activity of soil fauna. Mesofauna species feed on fungal hyphae and organic matter, contributing to nutrient cycling. The increased decomposition of maize residues by soil organisms led to higher substrate concentrations in the soil, resulting in increased microbial activity (Mcinga *et al.,* 2020; Rao *et al.*, 2017).

5.3.3 *β-glucosidase*

β-glucosidase is a common enzyme used to test soil health since it is quite common and predominant in soils. β-glucosidase releases the final product of glucose, an important carbon energy source of life to microbes in the soil (Das & Varma, 2010). In numerous soil health investigations, β-glucosidase is a favoured indicator due to its sensitivity to various management practices. It correlates with fungal activity, offering insights into biomass turnover and soil health (Rao *et al.,* 2017).

The β-glucosidase results were notably greater in the conservation and uncultivated systems compared to the conventional system. Our findings align with previous research by Pittarello *et al.* (2022), Galindo *et al.* (2020), Muzangwa *et al.* (2020) & Pheap *et al.* (2019), which have all demonstrated that incorporating crop residues, practising crop rotation, and applying no-till methods lead to increased β-glucosidase activity. Crop residues increase biomass-C input, boosting enzymatic activity, as highlighted in the study by (Hok *et al.,* 2018). According to Bera *et al.* (2017), β-glucosidase can maintain its maximum activity level until the crop's harvesting stage, as it plays a pivotal role in carbon mineralization. The increased levels noted in August 2022 can be linked to crop harvesting, which speeds up the breakdown of crop residues, resulting in the gradual buildup of soil organic matter over time (Bera *et al.,* 2017).

As residues are situated on the soil surface, they gradually release organic matter into the soil. Over time, in conjunction with root biomass and exudates, this slow release could potentially

lead to a noticeable increase in β-glucosidase activity in the subsoil layers, as observed in the study by Hok et al. in 2018. Nevertheless, the integration of livestock can have a beneficial impact by mechanically incorporating residues into the soil, thereby enhancing microbial activity. The moving of animals allows the transfer of minerals and organic matter between fields (Guesmi *et al.,* 2019). Our study shows that β-glucosidase was higher under grazing systems, and these findings are consistent with Galindo *et al.* (2023).

5.3.4 *Soil organic carbon (SOC)*

Soil organic carbon is a widely recognized indicator of soil health due to its influence on the soil's chemical, physical, and biological properties. It plays a fundamental role in numerous soil functions within agricultural soils, including nutrient cycling, forming soil aggregates, water retention, and providing habitats for biodiversity (Bongiorno *et al.,* 2019). Despite the significance of SOC, its reduction remains a primary concern for agricultural soils.

Elevated SOC results of the uncultivated and conservation system align with previous research findings (Koun *et al.,* 2023; Thapa *et al.,* 2023). The elevated levels of soil SOC can be attributed to the practice of crop rotation, as it affects the duration of biomass accumulation and leads to changes in soil nutrient availability, structure, and microbial characteristics (Koun *et al.,* 2023; Page *et al.,* 2020). Tillage is acknowledged for its role in decreasing soil organic carbon (SOC) as the cultivation process disrupts the soil, exposing organic matter that was previously encapsulated within soil macroaggregates to microbial decomposition.

Overall, SOC results were a bit lower than usual compared to results found by Koun *et al.* (2023), Muzangwa *et al.* (2020), and Patra *et al.* (2019). Koun *et al.* (2023) examined soil organic carbon sequestration in arid and semi-arid regions, discovering that systems involving cover crops experienced a decline in SOC levels over time. The lower SOC results in these systems can be attributed to the fact that in regions with severely depleted carbon content in their soils, particularly water-limited areas, there may be greater potential for the initial accumulation of SOC during the initial years. However, with time, the rate of SOC accumulation gradually diminishes until it reaches an equilibrium state (Koun *et al.,* 2023).

As previously mentioned in section 5.2.2, carbon-related results were higher in a grazing system. Thelen *et al.* (2010) explained the significance of including livestock in cropping systems in their study. The addition of manure from livestock and the inclusion of crop residues improved SOC. A greater portion of carbon derived from manure is preserved in the soil, likely

due to the fact that manure is partially decomposed and contains a higher percentage of chemically recalcitrant organic compounds (Adesoyin, 2020).

5.4 Incorporating enzymes and soil organic carbon into the soil health assessment

The importance of incorporating a combination of physical, biological, and chemical parameters to test soil health has been cited by many authors (Thoumazeau *et al.,* 2019; Griffiths *et al.,* 2016; Obriot, *et al.,* 2016; Kibblewhite *et al.,* 2008). It is evident that biological and biochemical factors are highly responsive to even minor alterations in soil conditions, mainly due to the crucial involvement of microorganisms in several essential biochemical processes. These processes involve the activities of soil microorganisms, responsible for cycling vital bio-elements (such as P, N, S, and C) and transferring energy within the soil ecosystem (Haney *et al.,* 2018; Karaca *et al.,* 2010).

This study integrated Biofunctool® parameters and soil enzymes into a PCA analysis (section 4.4). The observed positive correlation between the FDA and soil respiration suggests a direct link between the parameters and that they can be used to indicate the same soil function. Soil respiration is a multifaceted process that results from various sources of CO^2 within the soil. These sources can be broadly categorised into two main categories: autotrophic respiration, associated with root activity, and heterotrophic respiration, linked to the actions of soil microorganisms (Thoumazeau *et al.,* 2017). Given that FDA is an enzyme involved in the decomposition of organic matter, and microorganisms play a significant role in this process, the correlation between FDA and SituResp is logical.

While numerous articles have elucidated how the use of individual biological indicators can provide insights into the overall soil health of a system (Fierer *et al.,* 2021; Das & Varma, 2010; Karaca *et al.,* 2010), it is paramount to adopt an integrative approach that encompasses all relevant parameters (Creamer *et al.,* 2022). This comprehensive consideration allows for a more accurate assessment of soil health. In their study, Creamer *et al.* (2022) emphasised that most soil processes arise from the intricate interactions among biological, chemical, and physical attributes. For instance, the stability of aggregates is influenced by factors such as macrofaunal activity and soil texture (Lehman *et al.,* 2020). Therefore, it is crucial to account for environmental conditions (Creamer *et al.,* 2022; Chivenge *et al.,* 2007). Nonetheless, our research findings revealed that when employing Biofunctool® indicators, the soil quality index pointed to soil structure maintenance as the predominant factor influencing soil health rather than carbon transformation, where enzymes are primarily involved. This suggests that relying

solely on enzymes may not be the optimal method for assessing overall soil health. A more extensive study with a larger sample size is warranted to validate these results. However, the results, which solely focused on enzyme activity, exhibited a similar trend as the one found with Biofunctool® parameters. Enzyme activity was lowest in the conventional system and highest in the uncultivated system.

5.5 Conclusion

The conservation agricultural system offers distinct benefits compared to conventional agricultural management systems. Its capacity to lower input expenses, enhance soil's physical, chemical, and biological characteristics, and boost yields is especially known, with the potential to support sustainable intensification in numerous cases. The findings in our studies, for both the Biofunctool® and enzymes, highlighted a discernible gradient of soil disturbance, ranging from the conventional system to the conservation system and finally to an uncultivated field characterised by minimal disturbance. The study highlighted the impact of various farming practices and seasons on soil functions. The soil quality index revealed that structural maintenance had the most significant influence on the overall outcomes. This is the first Biofunctool® application in South Africa under different farming systems. All the tested indicators improved under a CA system, with uncultivated fields having the highest results. By incorporating functional indicators, the Biofunctool® index effectively captures the intricate nature of the soil system and its reactions to various farming practices. The study of enzymes, SOC, and active carbon also revealed a clear continuum of soil disturbance, spanning from the conventional system to the conservation system and ultimately to an uncultivated field characterised by minimal disruption.

Chapter 6: Concluding Chapter

6.1 Testing of hypotheses

The study aimed to evaluate soil health within farmers' Conservation Agriculture (CA) systems across various agroecosystem regions. This assessment was conducted by employing the Biofunctool® index and measuring the activity of specific soil enzymes. The investigation encompassed the examination of three distinct systems – conventional, conservation, and uncultivated fields – across two agroecosystems in South Africa, namely the North-West province and the Free-state province. The research goals were attained by successfully fulfilling the study's objectives, and the hypotheses were substantiated. To provide a concise overview, the hypotheses are outlined and elaborated upon below:

1. Conservation agriculture fields managed by application of no-tillage, residue retention and crop rotation will have greater Biofunctool® values.

There is ample documentation supporting the use of Conservation Agriculture for enhancing soil health (Page *et al.,* 2020; Thierfelder *et al.,* 2015; van Antwerpen *et al.,* 2021). In our research, the utilisation of Biofunctool® yielded significant findings regarding how conservation systems can positively impact soil health, particularly when closely associated with uncultivated systems. These conclusions align with existing literature trends that highlight the enhancement of soil health in Conservation Agriculture (CA) systems, specifically in South Africa (Gura *et al.,* 2022; Muzangwa *et al.,* 2020; Nortje & Laker, 2021; van Antwerpen *et al.,* 2021). The primary objective of CA is to rehabilitate degraded systems and promote soil health. We opted to use uncultivated fields as they remain in their natural state, free from agricultural disturbance. Our results clearly demonstrated a close correlation between CA systems and uncultivated fields, indicating that the implementation of CA does indeed enhance soil health compared to conventional systems. Therefore, our initial hypotheses can be validated.

2. Conservation agriculture fields managed by the application of no-tillage, residue retention and crop rotation will present increased soil enzyme activity.

Changes in soil management practices have led to the suggestion that soil biological parameters could serve as early indicators of the soil's health status (Lehman *et al.,* 2015; Rao *et al.,* 2017). Similar patterns were observed in enzyme activity, as were the Biofunctool®

results. Enzyme activity exhibited a notable increase in CA systems when compared to conventional fields. As anticipated, uncultivated fields showed higher enzyme activity, and there is a strong correlation between CA systems and these uncultivated fields. This suggests that under a regimen of no-tillage, residue retention, and crop rotation, enzyme activity does indeed increase, affirming the validity of our hypothesis.

3. Soil enzymes will be identified as single-use indicators for soil health assessment.

The activity of specific soil enzymes can provide valuable information about the biological and biochemical processes occurring in the soil (Karaca *et al.,* 2010). Enzymes play a pivotal role in a range of soil functions, encompassing nutrient cycling, organic matter decomposition, and the breakdown of contaminants. By gauging enzyme activity, researchers and farmers can glean valuable insights into the soil's functional capacity and overall health. Nevertheless, there is a growing emphasis on adopting a comprehensive approach to studying soil health, as chemical, physical, and biological processes influence the soil's condition. Enzyme analysis primarily falls under the chemical and biological processes, overlooking the physical properties (Fierer *et al.,* 2021; Karaca *et al.,* 2010). The soil quality index, as identified by the Biofunctool® indicators, underscored the significant influence of soil structure on soil health results, with less emphasis on chemical and biological properties. Although our results revealed similar trends, the Biofunctool® indicators and enzyme activity were notably lower in conventional systems than in conservation systems. This inconclusiveness in our hypothesis suggests that further sampling is required to draw definitive conclusions.

6.2 Recommendations to improve the study

A primary objective of sustainable agriculture should be the preservation of a broad spectrum of ecosystem services for future generations, with agricultural soils maintaining their multifunctional capacity. The term soil health is commonly used to encompass the soil's ability to provide various ecosystem functions and services. Effectively maintaining soil health requires setting benchmarks for acceptable levels of soil-based ecosystem functions. This involves carefully balancing functions that sustain food production with those supporting soil conservation, water quality, crop and livestock health, human well-being, and controlling greenhouse gas emissions.

While this study encompassed many of the soil indicators that impact soil health, there is a need to consider additional parameters. Firstly, for the Biofunctool®, a broader array of sampling sites should be included to obtain a more precise overall assessment of soil health. Factors such as soil texture, rainfall patterns, fertiliser application, and livestock grazing should also be taken into account in these studies to gain a comprehensive understanding of carbon and nitrogen mineralization rates. Evaluating yield capacity can offer valuable insights into the status of CA implementation.

Regrettably, there is a shortage of research or publications on critical topics such as cover crops, crop residues, soil water dynamics, soil biology, livestock integration, greenhouse gas emissions, and the various carbon fractions. Emphasizing the recommendation and adoption of suitable conservation agriculture approaches and technologies is crucial for diverse agroecosystems and communities.

References

Adam, G. & Duncan, H. 2001. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil biology and biochemistry*, 33(7-8): 943-951.

Adetunji, A.T., Lewu, F.B., Mulidzi, R. & Ncube, B. 2017. The biological activities of β-glucosidase, phosphatase and urease as soil quality indicators: a review. *Journal of soil science and plant nutrition*, *17*(3): 794-807.

Adhikari, K. & Hartemink, A.E. 2016. Linking soils to ecosystem services—a global review. *Geoderma,* 262: 101–111.

Allison, L. E. 1965. Organic Carbon. (*In* Black, C.A. *ed.* Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties. American society of agronomy, Madison. p. 1367-1378).

Babur, E. 2019. Effects of parent material on soil microbial biomass carbon and basal respiration within young, afforested areas. *Scandinavian journal of forest research,* 34(2): 94-101.

Basile-Doelsch, I., Balesdent, J. & Pellerin, S. 2020. Reviews and syntheses: The mechanisms underlying carbon storage in soil. *Biogeosciences,* 17: 5223-5242.

Bationo, A., Hartemink, A., Lungu, O., Naimi, M., Okoth, P., Smaling, E., Thiombiano, L. & Waswa, B. 2012. Knowing the African soils to improve fertilizer recommendations. (*In* Kihara, J., *ed. I*mproving soil fertility recommendations in Africa using the decision support system for agrotechnology transfer. Dordrecht: Springer. p. 19-42).

Bera, T., Sharma, S., Thind, H.S., Sidhu, H. & Jat, M. 2017. Soil biochemical changes at different wheat growth stages in response to conservation agriculture practices in a rice-wheat system of north-western India. *Soil research,* 56(1): 91-104.

Bieluczyk, W., Pereira, M., Guareschi, R., Bonetti, J., da Silva, G. & Neto, E. 2017. Soil carbon and nitrogen stocks, light organic matter, and training phosphorus under a crop-livestock integration system. *Semina: Ciencias Agrarias,* 38(4):125-1840.

Brady, N. C. & Weil, R. R. 2014. 14th ed. The nature and properties of soils. Edinburgh Gate: Pearson Education Ltd.

Bordy, E.M., Hancox, P.J. & Rubidge, B.S. 2005. The contact of the Molteno and Elliot formations through the main Karoo Basin, South Africa: a second-order sequence boundary. *South African journal of geology*, *108*(3): 351-364.

Buffaloe, M. 2020. Regenerative farming in South Africa. https://borgenproject.org/regenerative-farming-in-south-africa/ Date of access: 16 March 2023.

Canali, S. & Benedetti, A. 2003. Soil Nitrogen mineralization. (*In* Bloem, J., Hopkins, D.W. & Benedetti, A., *ed.* Microbial methods for assessing soil quality. Wallingford: CABI publishing. p. 127- 135).

Cregger, M., McDowell, N., Pangle, R., Pockman, W. & Classen, A. 2014. The impact of precipitation change on nitrogen cycling in a semi-arid ecosystem. *Functional ecology,* 28(6): 1534-1544.

Das, S.K. & Varma, A. 2010. Role of enzymes in maintaining soil health. (*In* Shukla, G. & Varma, A., *ed.* Soil enzymology. Heidelberg: Springer. p. 25-42).

Deng, S. & Popova, I. 2011. Carbohydrate hydrolases. (*In* Dick, R.P., *ed.* Methods of soil enzymology. Madison: Soil Science Society of America. (9): 185-209).

Dexter, A.R. & Czyz, E.A. 2000. Soil physical quality and the effects of management practices. (*In* Wilson, M.J., Maliszewska-Kordybach, B., *ed.* Soil quality, sustainable agriculture and environmental security in Central and Eastern Europe. Dordrecht: Springer. p. 153-165).

Dhehibi, B., Fouzai, A., Frija, A., Adhim, M.A., M'hamed, H.C., Ouerghemmi, H. & Rekik, M. 2023. Assessing complementary synergies for integrated crop–livestock systems under conservation agriculture in Tunisian dryland farming systems. *Frontiers in sustainable food systems,* 6: 1-18.

Cheng, Y.B. & Xia, Y.D. 2012. Soil microbial and enzymatic activities across a chronosequence of Chinese Pine plantation development on the Loess Plateau of China. *Pedosphere*, 22(1): 1–12.

Chivenge, P.P., Murwira, H.K., Giller, K.E., Mapfumo, P. & Six, J. 2007. Long-term impact of reduced tillage and residue management on soil carbon stabilization: Implications for conservation agriculture on contrasting soils. *Soil and tillage research*, 94(2): 328-337.

Creamer, R., Barel, G. & Bongiorno, G. & Zwetsloot, M.J. 2022. The life of soils: Integrating the who and how of multifunctionality. *Soil biology and biochemistry,* 166: 1-15.

Culman, S.W., Snapp, S.S., Freeman, M.A., Schipanski, M.E., Beniston, J., Lal, R., Drinkwater, L.E., Franzluebbers, A.J., Glover, J.D., Grandy, A.S. & Lee, J., 2012. Permanganate oxidizable carbon reflects a processed soil fraction that is sensitive to management. *Soil science society of America journal*, *76*(2): 494-504.

du Plessis, M. 2019. Is dipe skeurploeg elke jaar nodig? *SA Grain,* 2019:54-57. https://sagrainmag.co.za/2019/08/01/is-diep-skeurploeg-elke-jaar-nodig/ Date of access: 21 May 2022.

FAO. 2022. Conservation agriculture. https://www.fao.org/3/cb8350en/cb8350en.pdf Date of access: 07 May 2023

FAO. 2010. Farming for the Future in Southern Africa: An Introduction to Conservation Agriculture. REOSA Technical Brief. https://www.fao.org/fileadmin/user_upload/emergencies/docs/FAO_REOSA_Technical_Brief_1__July_2010_.pdf Date of access: 21 Jan. 2022

Fierer, N., Wood, S. & Bueno de Mesquita. 2021. How microbes can, and cannot, be used to assess soil health. *Soil biology and biochemistry,* 153: 1-7.

Fiorini, A., Boselli, R., Maris, S., Santelli, S., Perego, A., Acutis, M., Brenna, S. & Tabaglio, V. 2020. Soil type and cropping system as drivers of soil quality indicators response to no-till: A 7-year field study. *Applied soil ecology,* 155: 1-8.

Franzluebbers, A.J. 2002. Soil organic matter stratification ratio as an indicator of soil quality. *Soil and tillage research*, 66(2):95–106.

Fu, B., Chen, L., Huang, H., Qu, P. & Wei, Z. 2021. Impacts of crop residues on soil health: a review. *Environmental pollutants and bioavailability,* 1(33): 164-173.

Galindo, F., Delate, K., Heins, B., Phillips, H., Smith, A. & Pagliari, H. 2020. Cropping system and rotational grazing effects on soil fertility and enzymatic activity in an integrated organic crop-livestock system. *Agronomy,* 10(6): 1-18.

Gavin-Centol, M., Serrano-Cernero, D., Montserrat, M., Meyer, S., Schue, S., Kundel, D., Fliessbach, A., Truu, J., Birkhofer, K., Sanchez-Moreno, S. & Moya-Lerano, J. 2023. Basic and applied ecology, 69: 49-59.

Gestel, C.A.M., Kruidenier, M. & Berg, M.P. 2003. Suitability of wheat straw decomposition, cotton strip degradation and bait-lamina feeding tests to determine soil invertebrate activity. *Biological Fertile Soils,* 37: 115–123.

Giller, K.E., Witter, E., Corbeels, M. & Tittonell, P., 2009. Conservation agriculture and smallholder farming in Africa: the heretics' view. *Field crops research*, *114*(1): 23-34.

Graham, E.B., Wieder, W.R., Leff, J.W., Weintraub, S.R., Townsend, A.R., Cleveland, C.C., Philippot, L. & Nemergut, D.R. 2014. Do we need to understand microbial communities to predict ecosystem function? A comparison of statistical models of nitrogen cycling processes. *Soil biology and biochemistry*, 68: 279–282.

Green, V.S., Stott, D.E. & Diack, M. 2006. Assay for fluorescein diacetate hydrolytic activity: optimization for soil samples. *Soil biology and biochemisty,* 38: 693–701.

Griffiths, B.S., Römbke, J., Schmelz, R.M., Scheffczyk, A., Faber, J.H., Bloem, J., Pérès, G., Cluzeau, D., Chabbi, A., Suhadolc, M. & Sousa, J.P. 2016. Selecting cost effective and policy-relevant biological indicators for European monitoring of soil biodiversity and ecosystem function. *Ecological Indicators*, *69*: 213-223.

Guesmi, H., Salem, H.B. & Moujahed, N. 2019. Integration of crop-livestock under conservation agriculture system. *Journal of new sciences,* 65(1): 4601-4065.

Guimarães, R.M.L., Ball, B.C. & Tormena, C.A. 2011. Improvements in the visual evaluation of soil structure. *Soil use and management,* 27: 395-403.

Gura, I., Mnkeni, P., Du Preez, C.C. & Barnard, J.H. 2022. Short-term effects of conservation agriculture strategies on the soil quality of a Haplic Plinthosol in Eastern Cape, South Africa. *Soil and tillage research,* 220: 1-13.

Haney, R.L., Haney, E.B., Smith, D.R., Harmel, R.D. & White, M.J. 2018.The soil health tool - Theory and initial broad-scale application. *Applied soil ecology*, *125*: 162-168.

Herrick, J.E., Whitford, W.G., Soyza, A.G., de Zee, J.W.V., Havstad, K.M., Seybold, C.A. & Walton, M., 2001. Field soil aggregate stability kit for soil quality and rangeland health evaluations. *Catena,* 44: 27–35.

Hobbs, P.R. 2007. Conservation agriculture: what is it and why is it important for future sustainable food production? *The Journal of Agricultural Science*, *145* (2): 127.

Hok, L., de Moraes Sá, J.C., Reyes, M., Boulakia, S., Tivet, F., Leng, V., Kong, R., Briedis, C., da Cruz Hartman, D., Ferreira, L.A. & Inagaki, T.M. 2018. Enzymes and C pools as indicators of C build up in short-term conservation agriculture in a savanna ecosystem in Cambodia. *Soil and tillage research*, *177*: 125-133.

Hurisso, T.T., Culman, S.W., Horwath, W.R., Wade, J., Cass, D., Beniston, J.W., Bowles, T.M., Grandy, A.S., Franzluebbers, A.J., Schipanski, M.E. & Lucas, S.T. 2016. Comparison of permanganate-oxidizable carbon and mineralizable carbon for assessment of organic matter stabilization and mineralization. *Soil science society of america journal*, *80* (5).

IUSS Working Group. 2015. International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106. FAO, Rome.

Jackson, M.C. 1992. A review of the late Archean volcano-sedimentary Dominion Group and implications for the tectonic setting of the Witwatersrand Supergroup, South Africa. *Journal of African earth sciences and the Middle East*, *15*(2): 169-186.

Jones, A., Breuning-Madsen, H., Brossard, M., Dampha, A., Deckers, J., Dewitte, O., Gallali, T., Hallett, S., Jones, R., Kilasara, M., Le Roux, P., Michéli, E., Montanarella, L., Spaargaren,

O., Thiombiano, L., Van Ranst, E., Yemefack, M., Zougmore, R., (eds.) 2013. Soil Atlas of Africa. European Commission, Publications Office of the European Union, Luxembourg.

Kassam, A., Friedrich, T. & Derpsch, R. 2018. Global spread of conservation agriculture. *International journal of environmental studies*, 2018: 1-23.

Kaur, G., Singh, G., Motavalli, P., Nelson, K., Orlowski, J. & Golden, B. 2020. Impacts and management strategies for crop production in waterlogged or flooded soils: A review. *Agronomy journal,* 112 (3): 1475-1501.

Kibblewhite, M.G., Ritz, K. and Swift, M.J. 2008. Soil health in agricultural systems. Philosophical *Transactions of the Royal Society B: Biological Sciences*, 363(1492): 685-701.

Karaca, A., Cetin, S.C., Turgay, O.C. & Kizilkaya, R. 2010. Soil enzymes as indication of soil quality. (*In* Shukla, G. & Varma, A., *ed.* Soil enzymology. Heidelberg: Springer. p. 119-148).

Kodzwa, J., Gotosa, J. & Nyamangara, J. 2020. Mulching is the most important of the three conservation agriculture principles in increasing crop yield in the short term, under sub humid tropical conditions in Zimbabwe. *Soil and tillage research*, 197: 1-9.

Koun, P., Filloux, T., Vernet, P., Filloux, T., Sar, V., Seng, V., Srimongkol, P., Tantachasatid, P., Reaksmey, S., Pheap, S., Tivet, F. & Thoumazeau, A. 2023. Early effects of conservation agriculture on soil organic carbon dynamics of Mollisols in Cambodia. *Soil use and management,* 2023: 1-14.

Kratz, W. 1998. The bait-lamina test. *Environmental Science and Pollution Research*, *5*(2): 94-96.

Kulagowski, R., Thoumazeau, A., Leopold, A., Lienhard, P., Boulakia, S., Metay, A., Sturm, T., Tixier, P., Brauman, A., Fogliani, B. & Tivet, F. 2021. Effects of conservation agriculture maize-based cropping systems on soil health and crop performance in New Caledonia. *Soil and tillage research*, *212*: 1-25.

Lal, R. 2015. Sequestering carbon and increasing productivity by conservation agriculture. *Journal of soil and water conservation,* 70(3): 55A-62A.

Laker, M.C. 2005. Appropriate plant nutrient management for sustainable agriculture in southern Africa. *Communications in soil science and plant analysis* 36 (1-3): 89–106.

Lassabatère, L., Angulo-Jaramillo, R., Soria Ugalde, J.M., Cuenca, R., Braud, I. & Haverkamp, R. 2006. Beerkan estimation of soil transfer parameters through infiltration experiments-BEST. *Soil science society of America journal*, *70*(2): 521-532.

Lawinsider. 2022. Legal definitions dictionary. https://www.lawinsider.com/dictionary. Date of access: 26 Sept. 2022.

Le Cadre, E., Kinkondi, M., Koutika, L., Epron, D. & Mareschal, L. 2018. Anionic exchange membranes, a promising tool to measure distribution of soil nutrients in tropical multispecific plantations. *Ecologival Indicators,* 94(1): 254-256.

Lee, M. & Gambiza, J. 2022. The adoption of conservation agriculture by smallholder farmers in southern Africa: A scoping review of barriers and enablers. *Journal of rural studies*, 92: 214-225.

Lehmann, J., Bossio, D.A., Kogel-Knabner, I. & Rillig, M.C. 2020. The concept and future prospects of soil health. *Nature reviews earth & environment,* 1: 544–553.

Loranger, G., Ponge, J.F., Imbert, D. & Lavelle, P. 2002. Leaf decomposition in two semi-evergreen tropical forests: influence of litter quality. *Biology and fertile soils,* 35: 247-252.

Madegwa, Y. & Uchida, Y. 2021. Land use and season drive changes in soil microbial communities and related functions in agricultural soils. *Environmental DNA,* 3(6): 1214-1228.

Mattauer, R., Thoumazeau, A., Le Gall, S., Soiron, A., Rakotondrazafy, N., Berard, A., Brauman, A. & Meziere, D. 2022. Soil health in temperate agroforestry: influence of tree species and position in the field. *Archives of agronomy and soil science,* 69(10):1-20.

Maynard, D.G., Kalra, Y.P. & Crumbaugh, J.A., 1993. Nitrate and exchangeable ammonium nitrogen. *Soil sampling and methods of analysis*, 1: 25-38.

Mcinga, S., Muzangwa, L., Janhi, K. & Mnkeni, P. 2020. Conservation agriculture practices can improve earthworm species richness and abundance in the semi-arid climate of Eastern Cape, South Africa. *Agriculture,* 10(12): 1-12.

Millennium Ecosystem Assessment. 2005. Ecosystems and Human Well Being: Synthesis. Island Press, Washington D.C.

Mo, A.S., Qiu, Z.Q., He, Q., Wu, H.Y. & Zhou, B. 2016. Effect of continuous monocropping of tomato on soil microorganism and microbial biomass carbon. *Communications in soil science and plant analysis, 47*(9): 1067-1077.

Mtyobile, M., Mnkeni, P. & Muzangwa, L. 2019. Tillage and Crop Rotation Effects on Selected Soil Chemical Properties and Wheat Yield in a Sandy Loam Oakleaf Soil in the Eastern Cape, South Africa. *International journal of agriculture and biology,* 21: 367-374.

Mucina, L. & Rutherford, M.C. (eds) 2010. (CD set) The vegetations of South Africa, Lesotho and Swaziland. Strelitzia 19. South African national biodiversity institute, Pretoria.

Muhammad, S., Usman, M., Wakeel, A., Cheema, S., Imran, A. & Muhammad, F. 2020. Terrestrial ecosystem functioning affected by agricultural management systems: A review. *Soil & tillage research,* 196: 1-11.

Mulimbi, W, Nalley, L.L., Strauss, J. & Ala-Kokko, K. 2023. Economic and environmental comparison of conventional and conservation agriculture in South African wheat production. *Agrekon*, 2023: 1-19.

Muzangwa, L., Mnkeni, P.N.S. & Chiduza, C. 2017. Assessment of conservation agriculture practices by smallholder farmers in the Eastern Cape province of South Africa. *Agronomy*, 7(3).

Muzangwa, L., Mnkeni, P. & Chiduza, C. 2020. The Use of Residue Retention and Inclusion of Legumes to Improve Soil Biological Activity in Maize-Based No-Till Systems of the Eastern Cape Province, South Africa. *Agricultural research,* 9: 66-76.

Nortje, G.P. & Laker, M.C. 2021. Soil fertility trends and management in Conservation Agriculture: a South African perspective. *South African journal of plant and soil,* 2021: 1-11.

Obriot, F., Stauffer, M., Goubard, Y., Cheviron, N., Peres, G., Eden, M., Revallier, Vieublé-Gonod, L. & Houot, S. 2016. Multi-criteria indices to evaluate the effects of repeated organic amendment applications on soil and crop quality. *Agriculture, ecosystems & environment*, *232*: 165-178.

Patra, S., Julich, S., Feger, K., Jat, M., Sharma, P. & Schwarzel, K. 2019. Effect of conservation agriculture on stratification of soil organic matter under cereal-based cropping systems. *Archives of agronomy and soil science,* 65(14): 2013-2028.

Page, K., Dang, Y. & Dalal, C. 2020. The ability of conservation agriculture to conserve soil organic carbon and the subsequent impact on soil physical, chemical, and biological properties and yield. *Frontiers in sustainable food systems,* 4:1-17.

Panklang, P., Thaler, P., Thoumazeau, A., Chiarawipa, R., Sdoodee, S. & Bruaman, A. 2022. How 75 years of rubber monocropping affects soil fauna and nematodes as the bioindicators for soil biodiversity quality index. *Soil & plant science*, *72*(1): 612-622.

Paustian, K., Parton, W.J. & Persson, J. 1992. Modelling soil organic matter in organic-amended and nitrogen-fertilized long-term plots. *Soil science society of America journal*, *56*(2): 476-488.

Pell, M., Stenström, J. & Granhall, U. 2003. Soil respiration. (*In* Bloem, J., Hopkins, D.W. & Benedetti, A., *ed.* Microbial methods for assessing soil quality. Wallingford: CABI publishing. p. 117- 126).

Pheap, S., Lefevre, C., Thoumazeau, A., Leng, V., Boulakia, S., Koy, R., Hok, L., Lienhard, P., Brauman, A. & Tivet, F. 2019. Multi-functional assessment of soil health under conservation agriculture in Cambodia. *Soil & tillage research*, 194: 1-9.

Pittarello, M., Chiarini, F., Menta, C., Furlan, L. & Carletti, P. 2022. Changes in Soil Quality through Conservation Agriculture in North-Eastern Italy. *Agriculture*, *12*(7): 1-12.

Pittelkow, C.M., Liang, X., Linquist, B.A., Van Groenigen, K.J., Lee, J., Lundy, M.E., Van Gestel, N., Six, J., Venterea, R.T. & Van Kessel, C. 2015. Productivity limits and potentials of the principles of conservation agriculture. *Nature*, *517*(7534): 365-368.

Prosser, J.A., Spier, T.W. & Stott, D. E. 2011. (*In* Dick, R.P., *ed.* Methods of soil enzymology. America: SSSA. p. 68-84.)

Qian, P. & Schoenau, J.J. 2002. Practical applications of ion exchange resins in agricultural and environmental soil research. *Canadian journal of soil science*, *82*(1): 9-21.

Ranaivoson, L., Naudin, K., Ripoche, A., Affholder, F., Rabeharisoa, L. & Corbeels, M. 2017. Agro-ecological functions of crop-residues under conservation agriculture. A review. *Agronomy for sustainable development,* 37(26): 1-17.

Rao, C.S., Grover, M., Kundu, S. & Desai, S. 2017. Soil enzymes. *Enzymes in agricultural sciences,* 3: 2100-2107.

Riah, W., Laval, K., Laroche-Ajzenberg, E., Mougin, C., Latour, X. & Trinsoutrot-Gattin, I. 2014. Effects of pesticides on soil enzymes: a review. *Environmental chemistry letters*, *12*(2): 257-273.

Saccá, M.L., Barra Caracciolo, A., Di Lenola, M. & Grenni, P. 2017. Ecosystem services provided by soil microorganisms. (*In* Lucak, M., *ed.* Soil biological communities and ecosystem resilience. Switzerland: Springer International Publishing. p. 9-24)

Sanaullah, M., Usman, M., Wakeel, A., Cheema, S.A., Ashraf, I. & Farooq, M. 2020. Terrestrial ecosystem functioning affected by agricultural management systems: A review. *Soil and tillage research*, 196: 1-11.

Sanderson, M.A., Archer, D., Hendrickson, J., Kronberg, S., Liebig, M., Nichols, K., Schmer, M., Tanaka, D & Aguilar, J. 2013. Diversification and ecosystem services for conservation agriculture: outcomes from pastures and integrated crop–livestock systems. *Renewable agriculture and food systems*, *28*(2): 129-144.

Schimel, J.P. 2018. Life in dry soils: Effects of drought on soil microbial communities and processes. *Annual Review of ecology, evolution, and systematics,* 49: 409-432.

Siebert, J., Sunnemann, M., Auge, H., Berger, S., Cesarz, S., Ciobanu, M., Guerrero-Ramírez, N.R. & Eisenhauer, N. 2019. The effects of drought and nutrient addition on soil organisms

vary across taxonomic groups, but are constant across seasons. *Scientific reports*, 9(1): 1–12.

Sithole, N.J., Magwaza, L.S. & Thibaud, G.R. 2019. Long-term impact of no-till conservation agriculture and N-fertilizer on soil aggregate stability, infiltration and distribution of C in different size fractions. *Soil and tillage research,* 190: 147-156.

Shah, K., Modi, B., Pandey, H., Subedi, A., Aryal, G., Pandey, M. & Shrestha, J. 2021. Diversified crop rotation: An approach for sustainable agriculture production. *Advances in agriculture,* 2021: 1-9.

Shaw, L. & Burns, G. 2003. Enzyme activity profiles and soil quality. (*In* Bloem, J., Hopkins, D. & Benedetti, A., *ed.* Microbiological methods for assessing soil quality. United Kingdom: CABI Publishing. P. 158-171).

Shepherd, T.G. 2000. Visual soil assessment: field guide for cropping. https://orgprints.org/id/eprint/30582/1/VSA_Volume1_smaller.pdf Date of access: 25 June 2022.

Sherbine, K., Frankl, A., Fernandez, F., Pease, L. & Cates, A. 2023. Haney soil health test changes with season, not subsurface drainage. *Agricultural and environmental letters* 8(1): 1-7.

Strauss, J.A., Swanepoel, P.A., Smith, H. & Smit, E.H. 2021. A history of conservation agriculture in South Africa. *South African journal of plant and soil,* 38(3): 196-201.

Subler, S., Blair, J. M. & Edwards, A. C. 1995. Using anion exchange membranes to measure soil nitrate availability and net nitrification. *Soil biology and biochemistry,* 27: 911–917.

Sumberg, J. & Giller, K.E. 2022. What is 'conventional' agriculture? *Global food security, 32:* 1-9.

Tabatabai, M.A. 1983. Soil enzymes. (*In* Page, A.L. *ed.* Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties. American Society of Agronomy. p. 903-947).

Tal, A. 2018. Making conventional agriculture environmentally friendly: moving beyond the glorification of organic agriculture and the demonization of conventional agriculture. *Sustainability*, *10*(4): 1078.

Thapa, V.R., Ghimire, R., Adhikari, K.P. & Lamichane, S. 2023. Soil organic carbon sequestration potential of conservation agriculture in arid and semi-arid regions: A review. *Journal of arid environments,* 217: 1-7.

Thelen, K.D., Fronning, B.E., Kravchenko, A., Min, D.H. & Robertson, G.P. 2010. Integrating livestock manure with a corn–soybean bioenergy cropping system improves short-term carbon sequestration rates and net global warming potential. *Biomass and bioenergy,* 34: 960-966.

The South African Weather Service. Historical rain maps. 2022. https://www.weathersa.co.za/home/historicalrain. Date of access: 30 Sept. 2022.

Thierfelder, C., Baudron, F., Setimela, P., Nyagumbo, I., Mupangwa, W., Mhlanga, B., Lee, N. & Gérard, B. 2018. Complementary practices supporting conservation agriculture in southern Africa. A review. *Agronomy for sustainable development*, *38*(2): 1-22.

Thierfelder, C., Cheesman., S. & Rusinamhodzi, L. 2016. Benefits and challenges of crop rotations in maize- based conservation agriculture (CA) cropping systems of southern Africa. *International journal of agricultural sustainability,* 11(2): 108-124.

Thierfelder, C., Rusinamhodzi, L., Ngwira, A.R., Mupangwa, W., Nyagumbo, I., Kassie, G.T. & Cairns, J.E. 2015. Conservation agriculture in Southern Africa: Advances in knowledge. *Renewable agriculture and food systems*, *30*(4): 328-348.

Thomas, F., Rossi, J.P., Decaëns, T., Grimaldi, M., Lavelle, P., Fernando da Silva Martins, P. & Garnier-Zarli, E. 2008. Comparative analysis of Andiodrilus pachoensis casts in forests and pastures of South-Eastern Amazon. *European journal of soil biology*, 44: 545–553.

Thoumazeau, A., Gay, F., Alonso, P., Suvannang, N., Phongjinda, A., Panklang, P., Chevallier, T., Bessou, C. & Brauman, A. 2017. SituResp®: A time-and cost-effective method to assess basal soil respiration in the field. *Applied soil ecology*, *121*: 223-230.

Thoumazeau, A., Bessou, C., Renevier, M.S., Panklang, P., Puttaso, P., Peerawat, M., Heepngoen, P., Polwong, P., Koonklang, N., Sdoodee, S. & Chantuma, P. 2019. Biofunctool® Protocols. https://natres.psu.ac.th/pisai/document/module2/Protocols%20Biofunctool.pdf Date of access: 22 May 2022.

Thoumazeau A, Bessou C, Renevier MS, Trap J, Marichal R. & Mareschal L. 2019. Biofunctool®: a new framework to assess the impact of land management on soil quality. Part A: concept and validation of the set of indicators. *Ecological indicators*, 97:100-110.

Thoumazeau, A., Bessou, C., Renevier, M.S., Panklang, P., Puttaso, P., Peerawat, M., Heepngoen, P., Polwong, P., Koonklang, N., Sdoodee, S. & Chantuma, P. 2019. Biofunctool®: a new framework to assess the impact of land management on soil quality. Part B: investigating the impact of land management of rubber plantations on soil quality with the Biofunctool® index. *Ecological Indicators*, 97:429-437.

Trivedi, P., Delgado-Baquerizo, M., Anderson, I.C. & Singh, B.K. 2016. Response of soil properties and microbial communities to agriculture: implications for primary productivity and soil health indicators. *Frontiers in plant science*, *7*: 990.

Valbuena, D., Erenstein, O., Tui, S.H.K., Abdoulaye, T., Claessens, L., Duncan, A.J., Gérard, B., Rufin M.C., Teufel, N., van Rooyen, A. & van Wijk, M.T. 2012. Conservation agriculture in mixed-crop–livestock systems: scoping crop residue trade-offs in Sub-Saharan Africa and South Asia. *Field crops research*, *132*: 175-184.

van Antwerpen, R., Laker, M.C., Beukes, D.J., Botha, J.J., Collet, A. & du Plessis, M. 2021. Conservation agriculture farming systems in rainfed annual crop production in South Africa. *South African journal of plant and soil,* 38(3): 202-216.

van Gestel, C.A., Kruidenier, M. & Berg, M.P. 2003. Suitability of wheat straw decomposition, cotton strip degradation and bait-lamina feeding tests to determine soil invertebrate activity. *Biology and fertility of soils*, *37*(2): 115-123.

Van Oudenhoven, A.P., Petz, K., Alkemade, R., Hein, L. & de Groot, R.S. 2012. Framework for systematic indicator selection to assess effects of land management on ecosystem services. *Ecological indicators*, 21:110-122.

Vorobeichik, E.L. & Bergman, I.E. 2020. Bait-Lamina in the assessment of polluted soils: Choice of exposure duration. *Russian journal of ecology,* 51(5): 430-439.

WeatherSpark. 2022a. Climate and average weather year round in Delareyville, South Africa. https://weatherspark.com/y/91706/Average-Weather-in-Delareyville-South-Africa-Year-Round. Date of access: 26 Sept. 2022

WeatherSpark. 2022b. Climate and average weather year round in Ladybrand, South Africa. https://weatherspark.com/y/94180/Average-Weather-in-Ladybrand-South-Africa-Year-Round. Date of access: 26 Sept. 2022

Weil, R., Islam, K. R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E. 2003. Estimating active carbon for soil quality assessment: A simple method for laboratory and field use. *Alternative agriculture*, 18(1):3-17.

Wheldon, M.C., Anderson, M.J. & Johnson, B.W. 2007. Identifying treatment effects in multi-channel measurements in electroencephalographic studies: multivariate permutation tests and multiple comparisons. *Australian & New Zealand journal of statistics*, 49(4): 397-413.

Yang, H., Ma, J., Rong, Z., Zeng, D., Wang, Y., Hu, S., Ye, W. & Zheng, X. 2019. Wheat straw return influences nitrogen-cycling and pathogen associated soil microbiota in a wheat-soybean rotation system. *Frontiers in microbiology,* 10(667): 1-15.

Yang, G., Wagg, C., Veresoglou, S.D., Hempel, S. & Rillig, M.C. 2018. How soil biota drive ecosystem stability. *Trends in plant science*, 23(12):1057-1067.

Ward, P.S., Bell, A.R., Droppelmann, K. & Benton, T.G. 2018. Early adoption of conservation agriculture practices: Understanding partial compliance in programs with multiple adoption decisions. *Land use policy*, *70*: 1-32.

Zuber, S.M., Behnke, G.D., Nafziger, E.D. & Villamil, M.B. 2017. Multivariate assessment of soil quality indicators for crop rotation and tillage in Illinois. *Soil and tillage research*, *174*: 147-155