



Evaluating the soil health status of conservation and regenerative agricultural systems across multiple ecotopes in South Africa

J Brittion

 orcid.org/0000-0001-9093-3688

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

Supervisor: Prof GC du Preez
Co-supervisors: Dr PD Ramphisa-Nghondzweni
Co-supervisors: Dr L Muzangwa

Graduation: July 2025

ACKNOWLEDGEMENTS

I give all glory, honour, and praise to God Almighty, whose presence, guidance, and wisdom have been my strength throughout this journey. His unfailing love has sustained me through challenges, and His grace has provided me with the knowledge and perseverance to complete this study.

I would like to express my deepest gratitude to my beloved girlfriend, Denise Janse van Rensburg, for her unwavering support, encouragement, and love throughout this journey. Your belief in me has been a source of strength, and your patience and kindness have kept me motivated even in the most challenging moments. To my family, thank you for your endless prayers and sacrifices. This achievement is as much yours as it is mine.

I would like to extend my heartfelt gratitude to my main supervisor, Prof. Gerhard du Preez, for his invaluable guidance, patience, and expertise throughout this journey. Your mentorship has been instrumental in shaping my research, and I deeply appreciate the time and effort you dedicated to ensuring my growth and success. A special thank you to Dr. Lindah Muzangwa and Dr. Dimakatso Ramphisa for their continuous support, insightful feedback, and encouragement. Your guidance and expertise have been crucial in refining my work. I would like to express my sincere gratitude to Dr. João Marcelo Silva for his invaluable assistance with the data analysis.

I would like to extend my sincere appreciation to Dr. Hendrik Smith (Asset Research) for his invaluable assistance in coordinating the trial sites. A heartfelt thank you to The Maize Trust of South Africa for funding this project.

Lastly, to the farmers participating farmers (Hendrik Odendaal, Nicol de Vos, and Nant Yzel). Your willingness to contribute your time, resources, and insights has been invaluable, and this research would not have been possible without your collaboration.

ABSTRACT

Conventional agriculture negatively impacts soil health in South Africa due to an over-reliance on synthetic fertilisers, intensive tillage, no or limited crop rotations, as well as heavy irrigation. In response to these challenges, conservation and regenerative agriculture offer more resource-efficient alternatives with the potential to mitigate soil health degradation. However, further research is needed to quantify and validate the impact of conservation and regenerative agriculture under varying South African environmental contexts. Therefore, this study aimed to assess the impact of conservation and regenerative agriculture on soil health across multiple ecotopes in maize production regions of South Africa and to identify cost-effective indicators of soil health status. On-farm trial sites included Nicol de Vos (Kinross, Mpumalanga), Hendrik Odendaal (Standerton, Mpumalanga), and Nant Yzel (Marquard, Maluti). The trial design included implementation of conventional, conservation, and regenerative agriculture. Each according to their unique implementation principles. The effect of these implementations was monitored across three consecutive cropping seasons (2020/2021 - 2021/2022 - 2022/2023). Soil samples were collected during the summer growing seasons of each cropping season. Whereafter soil for soil texture (a physical parameter) and permanganate-oxidisable carbon (POXC) (a biological parameter) were analysed at the North-West University (NWU). Additional parameters were analysed using the Haney Analysis at Ward Laboratories Inc. in the United States of America. This study utilised Permutational Multivariate Analyses of Variance (PERMANOVAs) and Principal Component Analyses (PCAs) to assess the effects of ecotopes, farming systems, and cropping season on soil health. Pairwise comparisons were conducted to identify significant differences ($p < 0.05$) in soil health parameters, while distance-based linear models were used to determine the best subset of proxy indicators for soil health. The results revealed that soil texture and volumetric aggregate stability were the physical parameters most affected by farming systems across all three cropping seasons. Chemical parameters most affected by farming systems during the first cropping season included copper, magnesium, iron, and pH. During the second cropping season sulphur, manganese, and pH were most affected by the farming systems. Whereas sodium, iron, and inorganic nitrogen were most affected by farming systems during the final cropping season. Results further revealed that organic matter was consistently identified to be the most affected by farming systems across all three cropping seasons. However, the soil health status remained largely unchanged in the short term (<5 years). Only the final cropping season (2022/2023) indicated an improvement in soil health at Nant Yzel, the ecotope that exhibited the poorest soil health at the start of the study. Furthermore, this study identified magnesium, organic matter, pH, sodium, potassium, microbial active carbon, and nitrogen (total and inorganic) as key indicators that can serve as proxies of soil health status. The improvement in soil health at Nant Yzel were attributed to the slight increase in organic matter. However, due to the time it takes for organic matter to accumulate, it is anticipated that a longer study duration would have revealed more conclusive results. Due to the strong correlation between organic matter accumulation

and the effects of conservation and regenerative agriculture on soil health, along with its identification as a key proxy indicator, it was recommended that organic matter be measured as a single proxy for soil health status.

Key words: conservation agriculture; regenerative agriculture, soil health, ecotopes, proxy indicators

TABLE OF CONTENTS

CHAPTER 1	INTRODUCTION	1
1.1	TOWARDS SUSTAINABLE AGRICULTURE IN SOUTH AFRICA	1
1.2	MONITORING SOIL HEALTH	2
1.3	PROBLEM STATEMENT	3
1.4	AIMS AND OBJECTIVES	3
1.5	HYPOTHESIS.....	3
CHAPTER 2	LITERATURE REVIEW	5
2.1	AGRICULTURE IN SOUTH AFRICA	5
2.2.	ENVIRONMENTAL IMPACTS ASSOCIATED WITH CONVENTIONAL AGRICULTURE	6
2.2.1.	Synthetic fertiliser	7
2.2.2.	Intensive tillage	8
2.2.3.	Monocropping	9
2.2.4.	Heavy irrigation.....	10
2.3.	CONSERVATION AND REGENERATIVE AGRICULTURE AND THEIR ENVIRONMENTAL BENEFITS.....	11
2.3.1.	Minimal soil disturbance.....	13
2.3.2.	Permanent soil cover	14
2.3.3.	Crop rotation.....	14
2.3.4.	Differentiating between conservation and regenerative agriculture	15
2.3.4.1.	Cover cropping	16
2.3.4.2.	Intercropping.....	18
2.3.4.3.	Livestock integration	19
2.4.	SOIL HEALTH	20
2.4.1.	Physical indicators	21
2.4.2.	Chemical indicators	23
2.4.3.	Biological indicators	24
2.5.	SOIL HEALTH ASSESSMENT AND SCORING	25
2.5.1.	Haney Analysis and Soil Health Score	26
2.5.2.	Best subset (proxy) indicators.....	28
2.6.	IMPORTANCE OF ECOTOPES AS A SPATIAL UNIT.....	28
2.7.	LITERATURE CONCLUSION.....	30

CHAPTER 3	METHODOLOGY	31
3.1.	TRIAL SITES	31
3.1.1.	Nicol de Vos	32
3.1.2.	Hendrik Odendaal	33
3.1.3.	Nant Yzel	34
3.2.	TRIAL DESIGN	35
3.2.1.	Conventional farming systems implementation information	39
3.2.1.	Conservation farming systems production information	40
3.2.1.	Regenerative farming systems production information	40
3.3.	SOIL SAMPLING METHODOLOGY	41
3.4.	SOIL SAMPLE PREPARATION	42
3.5.	ANALYSIS OF PHYSICAL PARAMETERS	42
3.5.1.	Soil texture	43
3.5.2.	Volumetric aggregate stability	43
3.6.	ANALYSIS OF CHEMICAL PARAMETERS	43
3.6.1.	Soil pH	44
3.6.2.	Soil nutrients	44
3.6.3.	Total Organic Carbon and Total Nitrogen	44
3.6.4.	Nitrogen and Phosphorus Calculations	44
3.7.	ANALYSIS OF BIOLOGICAL PARAMETERS	45
3.7.1.	Permanganate oxidisable carbon (POXC) analysis	45
3.7.2.	Soil organic matter	45
3.7.3.	Soil respiration (CO ₂ – C)	46
3.7.4.	Water Extractable Organic Carbon (WEOC) and Water Extractable Organic Nitrogen (WEON)	46
3.8.	DETERMINATION OF THE SOIL HEALTH SCORE	46
3.9.	STATISTICAL ANALYSIS OF DATA	47
CHAPTER 4	RESULTS	49
4.1.	INFLUENCE OF CROPPING SEASON, ECOTOPES AND FARMING SYSTEMS ON SOIL HEALTH PARAMETERS	49
4.2.	COMPARISON OF MAIN SOIL HEALTH PARARMETERS	52
4.2.1.	Physical parameters	52
4.2.2.	Chemical parameters	55

4.2.3.	Biological parameters	63
4.3.	COMPARISON OF SOIL HEALTH SCORES AND IDENTIFICATION OF PROXY INDICATORS.....	64
4.3.1.	Soil Health Score	65
4.3.2.	Proxy indicators of soil health	65
CHAPTER 5	DISCUSSION	68
5.1.	OVERVIEW OF KEY FINDINGS PREVALENT IN THIS STUDY	68
5.2.	IMPACT OF ECOTOPES ON SOIL HEALTH	68
5.3.	IMPACT OF IMPLEMENTED FARMING SYSTEMS ON SOIL HEALTH	70
5.4.	IMPACT OF CROPPING SEASON ON SOIL HEALTH	72
5.5.	SOIL HEALTH STATUS UNDER CONSERVATION AND REGENERATIVE AGRICULTURE	73
5.6.	USING BEST SUBSET (PROXY) INDICATORS IN SOIL HEALTH ASSESSMENT	73
CHAPTER 6	CONCLUSION.....	75
6.1.	TESTING OF HYPOTHESES	75
6.2.	FUTURE RECOMMENDATIONS	76
BIBLIOGRAPHY.....		78

List of Tables

Table 2.1.1: Primary crop production for commercial agricultural purposes of the 2019/2020, 2020/2021, and 2023/2024 growing seasons (DALRD, 2021; DALRD, 2022; OPDT, 2024)	6
Table 2.3.1: Extent ('000 ha) of adoption of conservation agriculture worldwide by country in 2008/09, 2013/14 and 2015/16 (Kassam et al. 2019)	12
Table 2.3.2: Basic parameters, crops, and the benefits thereof to consider when choosing cover crops (Sharma et al. 2018)	17
Table 2.4.1: Illustration of the amount and form of macronutrients in soil (Jones, 2012)	23
Table 2.5.1: Soil quality Assessment frameworks developed in different regions (Bünemann et al. 2018; Haney et al. 2018).	25
Table 3.1.1: Location details of the trial sites, including the implementing farmer, the nearest town, and the coordinates of each trial.	31
Table 3.1.2: Precipitation information recorded to establish the Nicol de Vos ecotope (Knot et al. 2022)	32
Table 3.1.3: The edaphic information recorded to establish the Nicol de Vos ecotope (Knot et al. 2022)	33
Table 3.1.4: Precipitation information recorded to establish the Hendrik Odendaal ecotope (Knot et al. 2022)	33
Table 3.1.5: The edaphic information recorded to establish the Hendrik Odendaal ecotope (Knot et al. 2022)	34
Table 3.1.6: Precipitation information recorded to establish the Nant Yzel ecotope (Knot et al. 2022)	35
Table 3.1.7: The edaphic information recorded to establish the Nant Yzel ecotope (Knot et al. 2022)	35
Table 3.2.1: Original layout of the trials set out in Mpumalanga (Hendrik Odendaal and Nicol de Vos) (HO and NV): summer cover crops (SCC), winter cover crops (WCC).....	36

Table 3.2.2: Original layout of the trials set out in Maluti (Nant Yzel) (NY): summer cover crops (SCC), winter cover crops (WCC).....	36
Table 3.2.3: Cultivar and plant population information for maize and soy production for each ecotope during three consecutive cropping seasons	37
Table 3.2.4: Consistent summer cover crop mixture as per components and application rates per hectare for each ecotope	38
Table 3.2.5: Conventional farming system total fertiliser application kg/ha ⁻¹ (N:P:K) for Nicol de Vos (NV), Hendrik Odendaal (HO), and Nant Yzel (NY) during each cropping season for the production of maize	39
Table 3.2.6: Regenerative farming system total fertiliser application kg/ha ⁻¹ (N:P:K) for Nicol de Vos (NV), Hendrik Odendaal (HO), and Nant Yzel (NY) during each cropping season for the production of maize	40

List of Figures

Figure 2.4.1: Summary of conservation agriculture adoption (Findlater et al. 2019).....	13
Figure 2.4.2: Diagram of grazing density points of treatments within an integrated annual cropping pasture (Liebig et al. 2011)	20
Figure 2.5.1: Representation of soil health characteristics (Thapa et al. 2021)	21
Figure 2.5.2: Soil textural classes represented on the textural triangle (Brady and Weil, 2017).22	
Figure 2.6.1: Different biome regions of South Africa (Palmer et al. 2005).....	29
Figure 3.2.1: Locality map of the three trial sites that represent the ecotopes in this study (Own Compilation)	31
Figure 3.4.1: A) Soil sampling 100 meters from the strip's start (Photo: Dr. Gerhard du Preez). B) Five samples per location including the centre point sample (Photo: Dr. Gerhard du Preez)	41
Figure 4.2.1: Principal Component Analysis (PCA) plot illustrating variation in measured physical, chemical, and biological soil health parameters during the first cropping season (2020/2021). Distinct colours denote the three ecotopes: Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY). Different shapes represent the conventional (Conv), conservation (Cons), and regenerative agriculture (Regen) systems, as well as the uncultivated veld (Uncult) system. Overlay plots illustrate the main physical, chemical, and biological parameters with the greatest correlations ($R^2 > 0.7$; $p < 0.05$ or below $R^2 < -0.7$; $p < 0.05$) with the recorded variation in soil health parameters.	50
Figure 4.2.2: Principal Component Analysis (PCA) plot illustrating variation in measured physical, chemical, and biological soil health parameters during the second cropping season (2021/2022). Distinct colours denote the three ecotopes: Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY). Different shapes represent the conventional (Conv), conservation (Cons), and regenerative agriculture (Regen) systems, as well as the uncultivated veld (Uncult) system. Overlay plots illustrate the main physical, chemical, and biological parameters with the greatest correlations ($R^2 > 0.7$; $p < 0.05$ or below $R^2 < -0.7$; $p < 0.05$) with the recorded variation in soil health parameters.	51
Figure 4.2.3: Principal Component Analysis (PCA) plot illustrating variation in measured physical, chemical, and biological soil health parameters during the third sampling cropping season (2022/2023). Distinct colours denote the three ecotopes: Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY). Different shapes represent the conventional (Conv), conservation (Cons), and regenerative	

agriculture (Regen) systems, as well as the uncultivated veld (Uncult) system. Overlay plots illustrate the main physical, chemical, and biological parameters with the greatest correlations ($R^2 > 0.7$; $p < 0.05$ or below $R^2 < -0.7$; $p < 0.05$) with the recorded variation in soil health parameters. 52

Figure 4.3.1: Pairwise comparisons ($p < 0.05$) of sand content (%) for all three cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$) differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively..... 53

Figure 4.3.2: Pairwise comparisons ($p < 0.05$) of clay content (%) for all three cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$) differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively..... 54

Figure 4.3.3: Pairwise comparisons ($p < 0.05$) of volumetric aggregate stability (%) for all three cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$) differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively..... 55

Figure 4.3.4: Pairwise comparisons ($p < 0.05$) of H3A copper (Cu) (parts per million; ppm) for all three cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$) differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively..... 56

Figure 4.3.5: Pairwise comparisons ($p < 0.05$) of H3A magnesium (Mg) (parts per million; ppm) for all three cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult)

system. Capital and lower-case letters denote significant ($p < 0.05$ differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively). 57

Figure 4.3.6: Pairwise comparisons ($p < 0.05$) of H3A iron (Fe) (parts per million; ppm) for all three cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$ differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively). 58

Figure 4.3.7: Pairwise comparisons ($p < 0.05$) of pH for all three cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$ differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively). 59

Figure 4.3.8: Pairwise comparisons ($p < 0.05$) of H3A manganese (Mn) (parts per million; ppm) across cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$ differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively). 60

Figure 4.3.9: Pairwise comparisons ($p < 0.05$) of H3A sulphur (S) (parts per million; ppm) across cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$ differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively). 61

Figure 4.3.10: Pairwise comparisons ($p < 0.05$) of H3A sodium (Na) (displayed as parts per million; ppm) across cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$ differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively). 62

Figure 4.3.11: Pairwise comparisons ($p < 0.05$) of H3A inorganic nitrogen (Inorg. N) (parts per million; ppm) across cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$) differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively.

63

Figure 4.3.12: Pairwise comparisons ($p < 0.05$) of organic matter (%) across cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$) differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively..... 64

Figure 4.4.1: Pairwise comparisons ($p < 0.05$) of the soil health for all three-cropping season, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$) differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively 65

CHAPTER 1 INTRODUCTION

1.1 TOWARDS SUSTAINABLE AGRICULTURE IN SOUTH AFRICA

Conventional agriculture, characterised by the intensive use of agrochemicals, excessive soil disturbance, and often monoculture cropping, has a significant environmental impact in South Africa. These practices can result in soil erosion, nutrient depletion, and environmental pollution (Brady & Weil 2017; Lal 2009; Sanaullah et al. 2020; Savci 2012; Sithole et al. 2016), leading to a decline in soil health both globally and in South Africa. According to Sarkar et al. (2020), the availability of arable land per capita was 0.41 ha in 1961 and is expected to reduce to 0.20 ha per capita by 2050 due to soil degradation. Sithole et al. (2016) confirm this for South Africa, reporting that in South Africa, approximately one-third of the surface land and around 40% of agricultural land have been degraded due to the cumulative impacts of conventional agricultural practices.

In response to these challenges, researchers and industry stakeholders propose that crop producers implement conservation and regenerative agriculture (Hobbs 2007; Sumberg & Giller 2022). Conservation agriculture is defined as a farming system that promotes minimum soil disturbance, maintenance of a permanent soil cover, and diversification of plant species (i.e., plant rotation) (FAO, 2023). Conservation agriculture's primary aim is to minimise the environmental impact of agricultural practices and conserve resources. Regenerative agriculture is often discussed in the same context, but with the addition of cover crops and livestock integration (Newton et al. 2020; Rhodes 2017). In turn, regenerative agriculture has a holistic approach to restoring (regenerating) soil health and agrarian ecosystem productivity, which also justifies that the implementation of regenerative agriculture systems may result in healthier soils as compared to conventional or conservation agriculture systems (Hobbs 2007; Sithole et al. 2016). These different agricultural systems are known to affect soil properties differently, influencing factors such as organic matter content, nutrient cycling, and soil structure (Hobbs, 2007; Sithole et al. 2016; Verhulst et al. 2010).

The benefits of conservation and regenerative farming systems have received considerable attention since the early 2000s. Incentives aimed at development and adoption included formal research (e.g., peer-reviewed articles, reports, and presentations) and farmer-led research (e.g., on-farm trials, farmer groups, and awareness events) (Strauss et al. 2021b). The maize industry has notably supported this movement, increasing publication outputs since 2010 (Swanepoel et al. 2018). Mulimbi et al. (2023) report that by 2020, at least one principle had been adopted on 25% of South Africa's cropland, with the Western Cape province leading, with a 51% annual adoption rate.

1.2 MONITORING SOIL HEALTH

Conservation and regenerative agriculture play a key role in promoting soil health, which is essential for sustainable food production and long-term agricultural viability (Doran, 2002). Soil health encompasses physical, chemical, and biological (i.e., organic matter percentage) attributes (Brady & Weil 2017). Fine et al. (2017) report that soil health assessment is an important indicator of sustainable land management. The conventional scope of soil health assessments has expanded on traditional testing, primarily (but not solely) focusing on chemical soil properties (e.g., soil pH and nutrient status). While this conventional approach has been valuable in agricultural settings, its narrow focus has contributed to the neglect of the physical and biological properties of soil.

More comprehensive soil health assessment methodologies, such as the Haney analysis, have been developed in recent years. Retaining the measurement of physical and chemical soil properties, while placing an increased focus on the measurement of soil biological properties. The Haney analysis (Soil Health Tool) is a framework that evaluates the soil's physical, chemical, and biological components and generates an integrated score (Haney et al. 2018). This tool is increasingly popular among farmers in South Africa as it provides valuable insights for improving crop production. However, it is costly (approximately R2,110.00 per sample) (www.soilhealthlab.co.za) and requires samples to be sent to the United States for analysis. Nevertheless, the demand for soil health assessments is anticipated to rise with the broader adoption of conservation and regenerative farming systems.

Literature suggests that a selected subset of soil health indicators can effectively assess soil health by directly measuring key physical, chemical, and biological parameters. These indicators include soil texture, volumetric aggregate stability, plant-available water capacity, plant-available macro- and micronutrients, and soil organic matter (Fine et al. 2017). By using subsets of most predictive indicators soil health assessments could be simplified. Fine et al. (2017) strongly advocate for multi-indicator assessments, stating that subsets offer the potential for simplified soil health assessments but should not be viewed as a replacement for comprehensive multi-indicator frameworks like the Haney analysis.

1.3 PROBLEM STATEMENT

Conservation and regenerative farming systems are promoted as a means to enhance soil health and combat environmental degradation (Newton et al. 2020; Sithole et al. 2016). While these practices show significant potential, further research with standardised definitions and assessment approaches are necessary to fully understand and validate their impact (Jayasinghe et al. 2023). Evaluating these farming systems over multiple cropping seasons is essential to identify trends and long-term effects on soil health, especially across varied South African ecotopes. Collecting data over successive cropping seasons offers a more accurate assessment of soil dynamics and a clearer picture of these practices' effectiveness in supporting sustainable agriculture in diverse environments (Sithole et al. 2016). Existing assessment tools, such as the Haney Analysis, make it possible to evaluate soil health comprehensively, though they are often time-intensive and resource-demanding. Thus, an additional need to the demand of research on conservation and regenerative farming systems' ability to promote and restore soil health would be to look for singular indicators (or smaller subsets) that can be used as cheaper and faster assessment methodologies, representing soil health status.

1.4 AIMS AND OBJECTIVES

The study aimed to assess the impact of conservation and regenerative agriculture on soil health across multiple ecotopes in maize production regions of South Africa and to identify cost-effective indicators of soil health status.

The objectives included:

- 1) Characterising the physical, chemical, and biological properties of soil health under conventional, conservation, and regenerative agriculture systems.
- 2) Evaluating and comparing soil health status across cropping seasons, ecotopes, and agricultural systems.
- 3) Identifying cost-effective, singular indicators that can serve as proxies of soil health status.

1.5 HYPOTHESIS

We hypothesise that physical, chemical, and biological properties associated with soil health will vary significantly between conventional, conservation, and regenerative agricultural systems across multiple ecotopes, with regenerative systems expected to exhibit better overall soil health.

Using soil health scores derived from the Haney analyses, we further hypothesise that specific proxy indicators, such as the macro – and micronutrient, as well as soil organic matter, will emerge as key subsets of soil health status, offering practical, singular metrics for assessing soil health across diverse agricultural systems.

CHAPTER 2 LITERATURE REVIEW

2.1 AGRICULTURE IN SOUTH AFRICA

The agricultural industry has undergone revolutionary changes, transitioning from animal-drawn cultivation systems to mechanised farming. This shift led to the development of specialised practices, including intensive tillage, the use of synthetic fertilisers, and precision seed selection for monoculture production (Perret & Payen, 2020). By the 1930s, these methods became synonymous with conventional farming systems and were widely adopted worldwide (Sithole et al. 2016). High investment, crop research, infrastructure development, market expansion, and supportive policies introduced conventional farming systems to developing countries, including South Africa (Pingali, 2012). Adoption was initially slow after its introduction to Africa, with only 27% of the continent utilising conventional methods by 1998. In response, the Consultative Group on International Agricultural Research (CGIAR) focused on increasing adoption rates in sub-Saharan Africa. By 2005, conventional agriculture, with its intensive tillage, synthetic fertiliser use, and monoculture practices, accounted for 70% of wheat production and 45% of maize production (Pingali, 2012).

Currently, the agricultural sector in South Africa is divided into commercial farming and rural production. The economy is primarily driven by intensive crop production, particularly in terms of land use. Of the country's total land area of 122 million hectares, 101 million hectares are designated for agriculture. Of this, 14.2 million hectares are used for commercial crop production, while 2.6 million hectares are allocated for communal cropping. The remaining land is primarily dedicated to livestock farming, with 72 million hectares used commercially and 12 million hectares for communal livestock farming, making it the largest land use in South Africa (Du Preez & Van Huyssteen, 2020).

South Africa's nine provinces experience a range of climates, from arid to temperate, sub-tropical, and Mediterranean (Waldner et al. 2017). The country receives erratic rainfall, with an average annual precipitation of less than 500 mm (Sithole et al. 2016). Due to this low rainfall, 30% of total crop production relies on heavy irrigation (Waldner et al. 2017). This low rainfall and dependency on irrigation adds pressure to maintain production levels because of the unsustainable nature and high costs of intensive irrigation practices.

To conceptualise crop production in South Africa, Table 2.1.1 represents commercial crop yields for three production seasons (2019/2020, 2020/2021 and 2023/2024) (DALRD, 2021; DALRD, 2022; OPDT, 2024). In many cases, the production of crops in Table 2.1.1 is based on a

consecutive mono-crop production strategy that includes one summer crop and one winter crop (characteristic of conventional agriculture) (Waldner et al. 2017).

Table 2.1.1: Primary crop production for commercial agricultural purposes of the 2019/2020, 2020/2021, and 2023/2024 growing seasons (DALRD, 2021; DALRD, 2022; OPDT, 2024)

Crop	2019/2020 Tons (t)	2020/2021 Tons (t)	2022/2023 Tons (t)
Summer crops:			
Maize	15 300 000	16 234 265	16 430 000
Sunflower seeds	788 500	677 240	720 000
Soybeans	1 245 500	1 890 450	2 770 000
Groundnuts	50 080	59 950	53 000
Sorghum	158 000	213 645	94 360
Winter crops:			
Wheat	2 120 000	2 257 205	1 050 000
Malting barley	588 000	331 100	377 000
Canola	165 200	197 000	236 300
Cereal oats	57 000	69 950	41 000

The largest area of land used for crop production in South Africa is dedicated to maize, followed by wheat. Commercial maize producers are estimated to occupy 2.7 million hectares of land, with the Free State and North-West provinces accounting for 75% of the country's maize production. More than half of the maize produced is for human consumption. Wheat is primarily grown in the Western Cape under winter rainfall conditions, with 360,000 hectares allocated to its production in 2021 (DALRD, 2021; DALRD, 2022; Waldner et al. 2017).

2.2. ENVIRONMENTAL IMPACTS ASSOCIATED WITH CONVENTIONAL AGRICULTURE

Conventional agriculture relies heavily on synthetic fertilisers, intensive tillage, monoculture or crop rotation only with cash crops, and heavy irrigation in low-rainfall areas (Perret & Payen, 2020; Sithole et al. 2016). The resulting impacts, such as soil degradation, environmental pollution, and biodiversity loss, are well-documented (Brady & Weil, 2017; Lal, 2009; Sanaullah et al. 2020; Savci, 2012; Sithole et al. 2016).

2.2.1. Synthetic fertiliser

Synthetic fertiliser is an anthropogenic substance applied primarily to the soil to supplement essential plant-available nutrients (Chandini et al. 2019). The major macro-nutrients are nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S) (Brady & Weil, 2017). Synthetic fertilisers contain sufficient proportions of available macro-nutrients and contribute toward increased crop production and enhanced agricultural activity rates. Crops with sufficient nutrients grow faster, yield better, and perform better than crops lacking sufficient nutrients (Savci, 2012). Unfortunately, the overuse of synthetic fertilisers, as typical in conventional agriculture, presents unwanted environmental implications (Chandini et al. 2019; Lal, 2009; Massah & Azadegan, 2016; Savci, 2012).

Crops use fewer nutrients than those applied with synthetic fertilisers (Chandini et al. 2019; Savci, 2012). Excess nutrients enter surrounding water bodies even with ideal application regimes through three primary transport systems. The first transportation system is drainage, defined by Brady and Weil (2017) as the frequency and duration of periods when the soil is free from saturation with water. In an agricultural environment, the producers often implement drainage patterns to prevent excessive water saturation, which can harm root development and reduce crop yields, but neglect the considerations related to nutrient loss and environmental pollution. The second transportation system is leaching, defined by Brady and Weil (2017) as removing nutrients in solution under the influence of gravity. This type of transportation could occur in a downward or sideways motion. The final form of transportation is known as surface runoff, where some of the precipitation or irrigation water is prohibited from entering soil pore spaces and water containing the soil nutrients is lost by surface flow (Brady & Weil, 2017).

Savci (2012) and Chandini et al. (2019) explain that with the application of synthetic fertiliser, 2-20 % N gets volatilised, and 15-25 % N reacts with the organic compounds found in soil, while only 50 % N is used by plants. This leaves 2-10 % N to be transported into water bodies through at least one of the mentioned nutrient transportation systems. If NO_3^- concentration exceeds 50 mg/L in human drinking water, it may cause gastric cancer and heart disease in adults. When children or infants are exposed to these NO_3^- levels in drinking water, methemoglobinemia (blue baby syndrome) might occur (Chandini et al. 2019; Savci, 2012). Another principal concern with high-level N concentrations in water is eutrophication, whereby the associated ecosystem is deprived of oxygen, killing marine life and effecting water pollution (Chandini et al. 2019). Furthermore, phosphate (PO_4^{3-}) enrichment in wetlands, ponds, and other water bodies causes overstimulation of aquatic organisms (Brady & Weil, 2017).

Implementation of synthetic fertiliser without proper management and planning furthermore generates inevitable harmful gasses (Chandini et al. 2019). Shoji et al. (2001) reported that synthetic fertilisers accounted for 60 % of anthropogenic nitrogen dioxide (N_2O) gasses in the atmosphere. This gas has a 120-year lifetime with a global warming potential 320 times higher than that of carbon dioxide (CO_2), which has a lifetime of 100 years. Savci (2012) stated that atmospheric N_2O has increased at a rate of 0.2-0.3 % per year. Also of concern is the oxidation of ammonia (NH_3) to nitric acid (HNO_3) from lands that received synthetic fertiliser. Chandini et al. (2019) and Savci (2012) state that together with industrial sources of sulphuric acid (H_2SO_4), the formation of acid rain could occur. Acid rain can be toxic for living organisms and destroy vegetated areas.

Soils possess a strong buffer capacity that delays the effect of synthetic fertiliser application (Savci, 2012). However, the overuse of synthetic fertilisers initiates degradation reactions that cause soil nutrient imbalances (Savci, 2012). Du Preez and Van Huyssteen (2020) report that the addition of synthetic N fertilisers is directly responsible for acidification in agricultural land, which is also the most important contributing factor to soil infertility. Common examples of acid soil infertility caused by nutrient imbalances are as follows. The reduction of sulphur (S) and nitrogen (N) caused by lower levels of soil organic matter (SOM), reduced amounts of calcium (Ca) and magnesium (Mg) due to leaching, increased solubility of metal cations, and resultant aluminium (Al), iron (Fe), and manganese (Mn) toxicities in acidic soils (Du Preez & Van Huyssteen, 2020). These toxic elements not only pollute the soil but also accumulate in growing crops and enter the food chain, which may be harmful to living organism's dependent on these crops (Chandini et al. 2019). Furthermore, the addition of synthetic fertilisers that cause acidification severely affects soil structure (Chandini et al. 2019). Malik et al. (2018) stated that soils with a pH below 6.2 lose the ability to accumulate soil organic carbon (SOC), which negatively affects the soil structure, defined by Brady and Weil (2017) as the arrangement of primary soil particles (sand, silt, and clay).

2.2.2. Intensive tillage

Tillage is the mechanical manipulation of soil, standard in the preparation of crop production that involves soil disturbance (Brady & Weil, 2017; Sithole et al. 2016). This mechanical manipulation of soil dates back at least 10 000 years (Hobbs, 2007). In recent times, mechanical performances have evolved to the extent that the term tillage is referred to as intensive tillage (Hobbs, 2007). Beneficial traits such as incorporating previous crop residue, seedbed preparation, and aeration

of SOM are linked to intensive tillage. Although there are benefits to intensive tillage (Conant et al. 2007; Hobbs, 2007), the damaging effects are evident. One damaging effect of intensive tillage includes oxidation of SOM when exposed to direct air (Du Preez & Van Huyssteen, 2020; Hobbs, 2007). This causes a decline in SOC, which often only reaches a new equilibrium after 17 years (Du Preez & Van Huyssteen, 2020). Conant et al. (2007) report that intensive tillage reduced soil carbon levels by 27 % compared to its natural state (before intensive tillage). Intensive tillage also exposes the bare surface, creating an environment for aggregate breakdown (Hobbs, 2007). Brady and Weil (2017) explain that aggregate breakdown can clog soil pore spaces, leading to less infiltration, increased surface runoff, and soil loss. Soil loss can also occur through wind erosion, where soil particles are blown away. In the western parts of the Free State Province, where intensive tillage is practiced, soil loss through wind erosion is reported to exceed 59 milligrams per hectare (mg/ha) in one year (Du Preez & Van Huyssteen, 2020). Furthermore, upon the breakdown of soil aggregates, exposed surface clay slakes dry out and cause surface crusting (Du Preez & Van Huyssteen, 2020; Jephitha et al. 2023). Surface crusting contributes to less infiltration, surface runoff, soil loss and struggling seedling emergence (Du Preez & Van Huyssteen, 2020).

2.2.3. Monocropping

Monoculture crop production (monocropping) is characterised by the continuous production of one crop on one field (Brady & Weil, 2017; Nel, 2005). Monocropping is currently part of a simplified cropping system covering large, cultivated areas where indigenous plants are removed to promote agriculture (He et al. 2019; Nel, 2005). This way of cultivation leads to loss of available plant resources, decreased biodiversity, and higher susceptibility to plant pests and diseases (Brady & Weil, 2017; He et al. 2019; Sanaullah et al. 2020; Sithole et al. 2017). This susceptibility is not coincidental, specific organisms tend to accumulate as one crop dominates an area. It gives rise to pests and diseases with enough available food resources to reproduce efficiently without any defence from repelling polyculture influences.

Monocropping necessitates the use of pesticides to control unwanted diseases and promote agricultural output (Aktar et al. 2009). Pesticides can be coated around the seeds or directly sprayed onto a target crop through different spraying techniques (Degrendele et al. 2022). Through these implementations, pesticides reach the environment and cause contamination (Degrendele et al. 2022; Muhammetoglu et al. 2010). Muhammetoglu et al. (2010) report that the effects of pesticides depend heavily on the environment in which it is implemented. In South

Africa, Degrendele et al. (2022) reported the presence of nine different pesticides in the soil and air of the Hex River Valley and 16 in Grabouw. Pesticides directly impact humans and the environment (e.g., soil, water, air quality, and toxic effects on non-targeted organisms) (Aktar et al. 2009).

Direct exposure to pesticides or consumption through food commodities can cause immune dysfunction, hormone disruption, reproduction abnormalities, and cancer (Aktar et al. 2009). Pesticides also diminish beneficial insects and kill constructive organisms such as birds and fish, disrupting the ecological balance (Aktar et al. 2009). The same consequence applies to pesticides found in soils. As the population of soil organisms diminishes, their capacity to decompose complex organic compounds reduces, resulting in a decline in soil organic matter (Aktar et al. 2009).

2.2.4. Heavy irrigation

Heavy irrigation practices can impact freshwater resources and contribute to soil degradation (Perret & Payen, 2020). While only 20 % of the global croplands are irrigated, it accounts for 45 % of global food production (Perret & Payen, 2020). This leads to certain drawbacks, including salt accumulation in soils (Payen et al. 2016; Perret & Payen, 2020; Rengasamy, 2006), often referred to as salinisation (Brady & Weil, 2017). Sodium (Na), Ca, and Mg are the major cations involved in salinisation, while chloride (Cl), sulphate (SO_4^{2-}), and carbonate (CO_3^{2-}) are the major anions (Payen et al. 2016). The extent of salinisation is referred to as soil salinity, which is the concentration of salts in the soil and is measured with electrical conductivity (EC) (Payen et al. 2016). Rengasamy (2006) reports that soil is considered saline if the EC exceeds 4 deci-siemens per metre (dS.m^{-1}). Soil salinity and the process of salinisation may cause production loss and irreversible soil loss (Perret & Payen, 2020). Regarding these effects, Brady and Weil (2017) specifically highlight soil dispersion caused by the accumulation of Na in soil. The soil structure breaks down because of the Na hydration size and the number of monovalent bonds needed to balance the negative charge on a colloidal surface. Another drawback associated with heavy irrigation is that the accumulation of salts causes alkalinisation (Perret & Payen, 2020). In the process of alkalinisation, excess Na and Ca ions are added to the soil through irrigation, in which case the naturally occurring CO_3^{2-} and bicarbonate (HCO_3^-) binds with either one of the ions to raise the $\text{pH} > 8.2$ (Brady & Weil, 2017). In these conditions, micronutrients tend to precipitate, and the production of crops tends to experience micronutrient deficiency (Brady & Weil, 2017).

Excess irrigation also causes soils to become waterlogged, leading to constrained agricultural production (Zeng et al. 2020). Waterlogging creates an anaerobic environment that limits root respiration and nutrient uptake ability. Waterlogged soils are also prone to surface runoff (Brady & Weil, 2017).

2.3. CONSERVATION AND REGENERATIVE AGRICULTURE AND THEIR ENVIRONMENTAL BENEFITS

In response to the challenges presented by conventional agriculture, Sithole et al. (2016) report that conservation agriculture is a resource-saving agricultural productivity system that aims to achieve acceptable profits, high and sustainable production capacity, all while minimising environmental effects. The system is built by integrating three primary principles: 1) implementing systematic crop rotation, 2) minimising the disturbance of soil, and 3) maintaining constant organic soil cover (García-Palacios et al. 2019; Hobbs, 2007; Sanauallah et al. 2020; Sithole et al. 2016). These three principles constitute the foundation of conservation agriculture. If implemented separately, then the system is not classified as conservation agriculture (Kassam et al. 2019).

Some related benefits include enhanced biological processes, sustainable agriculture production intensification, comparable and often higher yields than conventional agriculture practice, ecological stability, reduced soil erosion, improved water retention, and nutrient use efficiency (Kassam et al. 2019). However, short-term implementations often do not show significant changes in soil health, particularly in terms of SOC buildup and yield changes, the latter of which is more commonly influenced by climatic conditions over short periods. To effectively understand the effects and benefits of conservation agriculture and regenerative agriculture, long-term field trials (over 5 years) are ideally needed (Swanepoel et al. 2018).

The adoption of conservation agriculture is successful in many countries, as in some regions in South Africa (Kassam et al. 2019). The global adoption rates are presented in Table 2.3.1 as portrayed by Kassam et al. (2019) as the worldwide adoption of conservation agriculture by country, measured in thousands of hectares.

Table 2.3.1: Extent ('000 ha) of adoption of conservation agriculture worldwide by country in 2008/09, 2013/14 and 2015/16 (Kassam et al. 2019)

No.	Country	2008/2009	2013/2014	2015/2016
1	USA	26 500	35 613	43 204
2	Brazil	25 502	31 811	32 000
3	Argentina	19 719	29 181	31 028
4	Canada	13 418	18 313	19 936
5	Australia	12 000	17 695	22 299
6	Paraguay	2 400	3 000	3 000
7	Kazakhstan	1 300	2 000	2 500
8	China	1 330	6 670	9 000
9	Bolivia	706	706	2 000
10	Uruguay	655	1 072	1 260
11	Spain	650	792	900
12	South Africa	368	368	439
13	Germany	354	200	146
14	Venezuela	300	300	300
15	France	200	200	300

In South Africa, each of the fundamental principles that support conservation agriculture has an adoption rate exceeding 40%. The simultaneous adoption rate of all three principles was 14.2% in 2019 and revised to 25 % in 2020. Figure 2.3.1 summarises conservation agriculture adoption rates in South Africa (Findlater et al. 2019).

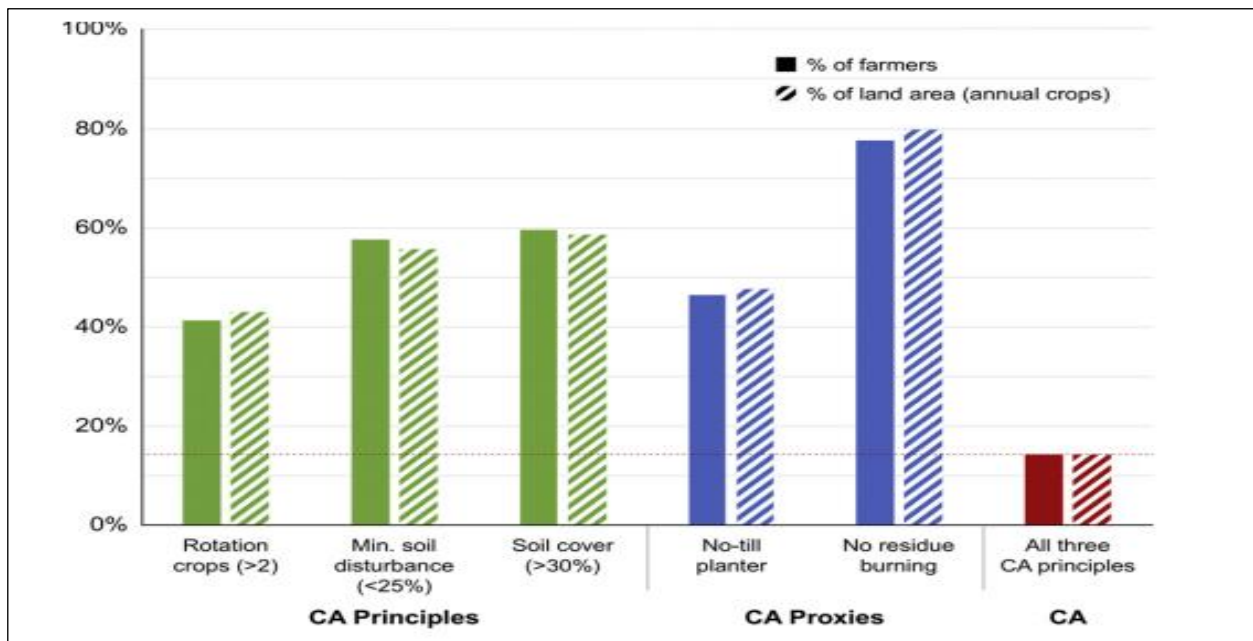


Figure 2.3.1: Summary of conservation agriculture adoption (Findlater et al. 2019)

Selective adoption of only one or two of the principles contrasts with conservation agriculture's comprehensive approach, reducing its full environmental benefits (Strauss et al. 2021a). The result of farmers' views of conservation agriculture as only no-till farming leads to the reason why implementing all three principles are lacking compared to singular characteristic implementation (Findlater et al. 2019). Understanding conservation agriculture starts with understanding each principle individually and then implementing all three simultaneously (Kassam et al. 2019).

2.3.1. Minimal soil disturbance

Minimal soil disturbance involves implementing minimum tillage, where soil disruption is limited primarily to planting activities (Bai et al. 2018). This principle has led to replacing or removing conventional equipment such as ploughs, disks, and cultivators with implements capable of leaving the soil relatively undisturbed, including no-till planters (Sithole et al. 2016). In contrast to conventional agriculture, where the aggregate formation is disrupted by intensive tillage, minimum tillage generally leads to SOM increases and promotes aggregate stability (Verhulst et al. 2010; Jephitha et al. 2023). These improvements can stabilise nutrient cycling, reduce bulk density, and improve soil water holding capacity, infiltration, and water use efficiency (Bai et al. 2018).

Carbon sequestration is a beneficial environmental response to higher organic matter and increased aggregate stability (Muzangwa et al. 2020). Gaskin et al. (2020) defined carbon

sequestration as the storage of carbon over periods that extend hundreds of years. By reducing mechanical disruption, soil structure is preserved, allowing for the formation and stabilisation of aggregates that protect SOC (Turmel et al. 2015). This process improves soil water retention and nutrient cycling while also supporting microbial activity essential for long-term soil fertility. Additionally, maintaining undisturbed soil minimises CO₂ emissions and promotes greater SOC sequestration, contributing to overall soil resilience and productivity (Lal, 2004; Muzangwa et al. 2020).

2.3.2. Permanent soil cover

Permanent soil cover, in the context of conservation agriculture, involves retaining crop residues after harvesting rather than leaving the soil bare. These residues form a protective layer of organic material on the field (Turmel et al. 2015). One of the key benefits of permanent soil cover is improved soil structure (Hiel et al. 2016), achieved through three primary mechanisms: (1) increased soil organic matter (SOM) and aggregate stabilisation, (2) protection against raindrop impact, and (3) reduced compaction caused by raindrop impact (Turmel et al. 2015). The incorporation of plant residues enhances SOM content, which acts as a binding agent between soil particles, improving aggregate stability and reducing susceptibility to erosion and compaction (Hiel et al. 2016; Six et al. 2000; Verhulst et al. 2010). By shielding the soil surface from direct raindrop impact, permanent soil cover minimises surface sealing and crust formation, which would otherwise restrict water infiltration and increase runoff (Turmel et al. 2015). Additionally, by reducing raindrop-induced compaction, permanent soil cover helps maintain a loose, well-aerated soil structure. Nonxuba et al. (2025) reported that crop residue management, as a form of permanent soil cover, positively influences soil bulk density and porosity in the upper 20 cm of soil in their study conducted in the Eastern Cape, South Africa (Turmel et al. 2015).

2.3.3. Crop rotation

Crop rotation is the sequential cultivation of different crops on the same field to maintain soil productivity, primarily by supporting plant growth. This approach contrasts with monocropping, where a single crop is continuously grown on the same land over multiple cropping seasons (Nel, 2005). One of the key benefits of crop rotation is its positive impact on soil water dynamics. It enhances water retention and storage capacity, improves water use efficiency in crops, and promotes water infiltration, thereby reducing the risk of surface runoff (Yu et al. 2022). These improvements create a more stable soil structure, fostering aggregate stability and reducing soil

compaction and erosion, which further supports overall soil health (Yu et al. 2022). Crop rotation also plays a critical role in balancing soil nutrient availability. Leguminous crops, such as soybeans, contribute significantly to nitrogen enrichment in the soil, reducing dependence on synthetic fertilisers (Nel, 2005). This process is facilitated through symbiotic nitrogen fixation, where legumes release compounds that attract rhizobia bacteria (e.g., *Rhizobium japonicum* and *Bradyrhizobium* spp.). These bacteria attach to the legume's root hairs, forming nodules where atmospheric nitrogen (N_2) is converted into ammonia (NH_3). The plant then assimilates NH_3 into usable nitrogen ions essential for growth. When the legume plants decompose, accumulated nitrogen is released into the soil, benefiting subsequent crops and enhancing fertility in a sustainable manner (Brady & Weil, 2017). The implementation of crop rotation also stimulates beneficial microbial activity in the soil. Microorganisms act as catalysts for enzyme-driven reactions, improving nutrient availability and soil fertility (Yu et al. 2022). Increased microbial diversity, microbial abundance, and a balanced bacteria-to-fungi ratio contribute to the stabilisation of microbial carbon and nitrogen content. This microbial activity is crucial in suppressing soil-borne diseases, as healthier soil conditions make plants more resilient to pathogens (Yu et al. 2022). Ouda et al. (2018) provides examples of disease reduction through crop rotation, including a two-year rotation of corn or winter wheat with soybean, which significantly reduced the formation of apothecia by *Sclerotinia sclerotiorum* in soybean fields compared to continuous soybean cultivation. Beyond soil and plant health, crop rotation also aids in weed management by disrupting weed life cycles and altering field conditions. A diverse crop rotation prevents any single weed species from becoming dominant, thereby reducing overall weed pressure (Yu et al. 2022). Additionally, certain crops, such as sorghum, eucalyptus, mulberry, and sunflower, exhibit allelopathic properties. These plants release chemical compounds that influence or inhibit the growth, development, and reproduction of competing weed species, further aiding in natural weed control (Shahzad et al. 2021).

2.3.4. Differentiating between conservation and regenerative agriculture

The common understanding is that regenerative and conservation agriculture have overlapping principles: minimal soil disturbance, permanent soil cover, and crop rotation (Newton et al. 2020). Conservation agriculture aims to minimise the environmental impact of agricultural practice and conserve resources. In comparison, regenerative agriculture has a holistic approach to restoring (regenerating) soil health and agriculture ecosystem productivity (Hobbs, 2007; Sithole et al. 2016). Additional practices associated with regenerative agriculture include integration of cover

crops and intercropping, as well as integration of livestock into the system (Hilimire, 2011; Lemaire et al. 2014; Sekaran et al. 2021).

2.3.4.1. Cover cropping

The Soil Science Society of America (SSSA) define cover crops as those used in the production system for a purpose other than harvesting, used to protect and improve soil between times of regular crop production (Koudahe et al. 2022; Smit et al. 2021). Cover cropping provides a wide range of benefits to soil health's physical, chemical, and biological properties (Koudahe et al. 2022; Sharma et al. 2018; Smit et al. 2021; Steenwerth & Belina, 2008). Physically, cover cropping provides protective cover to the soil by absorbing raindrop kinetic energy, thereby reducing aggregate detachment and runoff velocity. The opportunity for water to infiltrate soil is increased, and water-stable aggregates are promoted (Adetunji et al. 2020). In return, this further encourages soil hydraulic properties. Adetunji et al. (2020) report that in Arkansas (United States of America), silt loam and loam soil managed with crimson clover, winter rye and hairy vetch cover crops over 17 years increased soil absorbency, water retention capacity, and saturated hydraulic conductivity.

Studies have indicated that cover crop management with hairy vetch, crimson clover, pea, turnip Brassica, oats, and rye increases SOC concentrations compared to plots without cover crop management. Cover crop management with hairy vetch and crimson clover also indicated an increase in N concentration compared to fallow soils (Adetunji et al. 2020). The primary influence for increased C and N levels results from more organic matter plant residues and soil biota metabolism upon cover crop implementation (Brady & Weil, 2017).

Biologically, soil microbial activity (e.g., fungal hyphae, bacterial extracellular polysaccharides, and hydrophobic compounds) contributes to soil health by improved aggregation and nutrient cycling (Koudahe et al. 2022). The added SOM that cover crops provide within a system serves as a source of energy and nutrients, supporting the growth and activity of microorganisms. Microorganisms release enzymes into the soil, resulting in accelerated biochemical reactions, promotion of decomposition of crop residues, and the cycling of nutrients. This results in the release of inorganic nutrients essential for plant growth. The interaction between soil organic matter, microbial activity, and enzymatic processes forms the primary connection responsible for nutrient release to plants (Adetunji et al. 2020). Cultivating crimson clover, pea, wheat, and vetch can promote these benefits (Adetunji et al. 2020). From a study in continental Missouri, USA, it was found that the effects of a slight alteration (increased amounts) in the microbial communities

(e.g., total fungi, total bacteria, rhizobia, and actinomycetes biomass) from a corn-soybean rotation had quantifiable effects (beneficial) on the soil microbial biomass and community structure (Koudahe et al. 2022).

Considering the benefits that cover crops offer, selecting them based on their specific functions is essential. Due to the diverse roles of different cover crops, it is common to plant them in combinations (Sharma et al. 2018). Effective cover crops should possess specific qualities, including (but not limited to) rapid germination and emergence, adaptability to varying climatic conditions, nitrogen fixation capability, substantial biomass production, non-competitiveness with main crops, ease of management, resistance to pests and diseases, weed suppression, and cost-effectiveness (Sharma et al. 2018). The criteria for choosing appropriate cover crops are site-specific, although Table 2.3.2 provides some basic parameters for choosing cover crops with a specific benefit in mind.

Table 2.3.2: Basic parameters, crops, and the benefits thereof to consider when choosing cover crops (Sharma et al. 2018)

Parameters		Crops	Benefits
Weather conditions	Winter cover crop	- Winter rye - brassicas - hairy vetch - red clover - oats.	Controls erosion, source of nitrogen fixation, soil organic matter and improves soil structure.
	Summer cover crop	- Buckwheat - Sorghum Sudan grass - Cowpea, sun hemp	Increase soil organic matter, enhance soil microbes, weed suppression, and reduce soil erosion.
Type of cover crop	Legumes	- Hairy vetch - Peas - Red clover - Crimson Clover - Beans	Nitrogen fixation, prevent soil erosion, increase organic matter, helpful for insects and pollinators.
	Non-cover crops	- Wheat - Oats - Barley - Annual ryegrass - Brassicas, mustards - Buckwheat	Prevent soil erosion, nutrient scavenging, weed suppression, provide ground cover.

Grasses (Poaceae), legume cover crops (Leguminosae/Fabaceae), and non-legume broadleaf (Brassicaceae) cover crops are the most common options to integrate into an agriculture system (Sharma et al. 2018). Grasses are used for growth potential and slow decay, contributing to comprehensive soil cover and accumulative organic matter. Legumes are selected for their ability to fix atmospheric N with the nodules found in their roots, while non-legume broad leaves are typically used for deep soil penetration and improvement of soil structure and stability (Koudahe et al. 2022).

2.3.4.2. Intercropping

Intercropping is the simultaneous growth of two or more crops in the same row arrangement of the same field in a growing season (Cong et al. 2015; Jungers et al. 2019; Maitra et al. 2021; Yang et al. 2020). Intercropping can be implemented in different combinations that include (but are not limited to) annual and perennial crops depending on the producer's choice and the suitability of the growing conditions (Maitra et al. 2021). The different types of intercropping systems that are implemented globally can be categorised into the following options. Row intercropping involves planting one or more cash crops in evenly spaced rows and simultaneously cultivating the intercrops between them. This method is commonly aimed at efficiently utilising resources and maximising productivity through careful resource management (Maitra et al. 2021). Mixed intercropping is where two or more crops are grown without indication of row proportions. An example of this is grass-legume intercropping to enhance pastures in dairy farm systems (Maitra et al. 2021). Furthermore, strip intercropping is commonly used with two or more crops in strips on a particular land. The crops are cultivated perpendicular to the slope of the land or the direction of prevailing winds. This intercropping method is commonly used to maximise yield (Maitra et al. 2021). The final category of intercropping (most relevant to the trial sites, as it is the technique that was used) is relay intercropping – two or more crops are raised during a portion of the growing period. The second crop is seeded when the first crop has completed a significant portion of its growth cycle and reached a reproduction stage before harvesting (Maitra et al. 2021). As an example of intercropping, relay cropping actively contributes to soil health regeneration (Tanveer et al. 2017). It has the ability to reduce soil nutrient loss by taking up nutrients that would normally be leached out if no intercropping was done. For example, Tanveer et al. (2017) report on a study where the planting of wheat in standing maize retained 130 kg N/ha from the root zone and reproduced an extra 2-ton residue/ha that was integrated as soil cover. Additionally, leaving

the crop residue of the relay intercrops increases soil organic matter, improving soil health's physical, chemical, and biological characteristics (Tanveer et al. 2017).

Intercropping increases plant diversity and complementarity function, providing producers with higher yields than monocropping systems measured by land equivalent ratio. Essentially, less land is required to produce the same amount of product with the integration of intercropping than needed for monocropping systems (Cong et al. 2015). As an example, a study done in a semi-arid region of China found that by intercropping corn, wheat, and fababeans, 23 %, 4 %, and 11 % more root biomass, organic C, and N accumulated in the topsoil layer than those same species planted in conventional rotation (Cong et al. 2015). Producers should carefully select intercropping system species to optimise environmental benefits (Yang et al. 2020). For example, grass species tend to flourish in soils with high N availability, and legumes are known to fix N resulting from their symbiotic relationship with rhizobium bacteria. Therefore, grass-legume intercropping systems can act in self-regulation to improve soil N levels, soil nutrient use, and minimise the carbon footprint (Yang et al. 2020).

2.3.4.3. Livestock integration

Integrated crop-livestock systems provide an alternative strategy to conventional farming to minimise environmental damage and increase agricultural production (Hilimire, 2011; Lemaire et al. 2014; Sekaran et al. 2021). Hilimire (2011) reports that integrated livestock systems are only referred to as such when crop and livestock production is done on the same farmland. In these cases, crops and livestock are raised so that the product of one can be utilised by the other and vice versa. For example, Hilimire (2011) discusses this cycle where animal waste application contributes to crop production through nutrient acquisition, followed by cultivating forage crops for animal consumption. When correctly managed, the excreta in such a system present benefits of increased soil organic matter, more microbial biodiversity, increased macro-and micronutrients, reduced soil erosion, better crop quality and yield, and increased economic return (Clark, 2004; Hilimire, 2011; Lemaire et al. 2014; Sekaran et al. 2021).

Managing integrated crop-livestock systems to benefit a production system forms part of innovative regenerative grazing, specifically adaptive multi-paddock (AMP) grazing (Teague & Kreuter, 2020). This form of rotational grazing involves concentrating livestock on a single paddock at a time. There are multiple paddocks to which the producer may shift the concentrated livestock, providing sufficient time for other paddocks to regain their full potential. Liebig et al. (2011) report on a study where the management of an AMP includes control by electric fences.

The fences were moved daily to provide livestock (in the case of that study, it was cows) with access to fresh forage. As in Figure 2.3.2, the watering point was also placed at the edge of the occupied paddock, ensuring further densification of the integrated livestock.

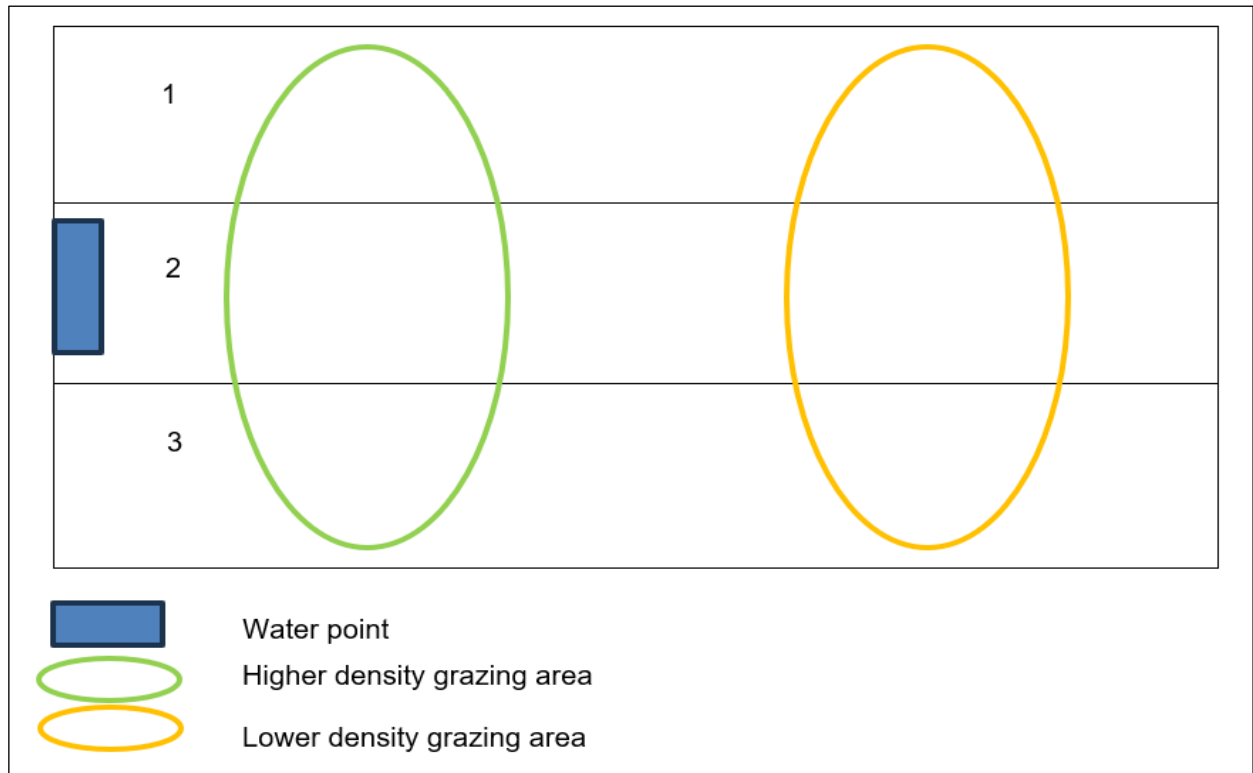


Figure 2.3.2: Diagram of grazing density points of treatments within an integrated annual cropping pasture (Liebig et al. 2011)

2.4. SOIL HEALTH

Soil represents a three-dimensional natural body within a complex ecosystem, capable of fulfilling numerous soil functions including 1) supporting plant growth, 2) regulating water supply, 3) recycling raw materials, 4) modifying the atmosphere, 5) sustaining habitats for various organisms, and 6) serving as a raw material for engineering applications such as earthen structures in civil projects (Brady & Weil, 2017). Therefore, soil health represents capacity of a soil to function as a system within the boundaries of an ecosystem or defined land use to sustain optimal productivity of plants and animals while enhancing or maintaining water and air quality, simultaneously promoting plant and animal health (Brady & Weil, 2017; Doran, 2002; Haney et al. 2018; Laishram et al. 2012; Yang et al. 2020). Promoting soil health is important in achieving more sustainable food production and is therefore a key outcome of conservation and

regenerative agriculture. To monitor the impact of agriculture systems on soil health status, assessment frameworks have been developed and generally include measurements of soil physical (i.e. soil texture and soil structure), chemical (i.e. pH level and fertility status), and biological (i.e. organic matter percentage) attributes (Brady & Weil, 2017).

It is important to recognise that soil health is an intrinsic property of soil defined by specific traits (physical, chemical, and biological), and that these traits frequently shift based on how the soil is utilised (e.g., agricultural purposes) (Haney et al. 2018; Lal, 2016). Effective management of soil health requires an understanding of the broad characteristics presented in Figure 2.4.1 (Thapa et al. 2021).

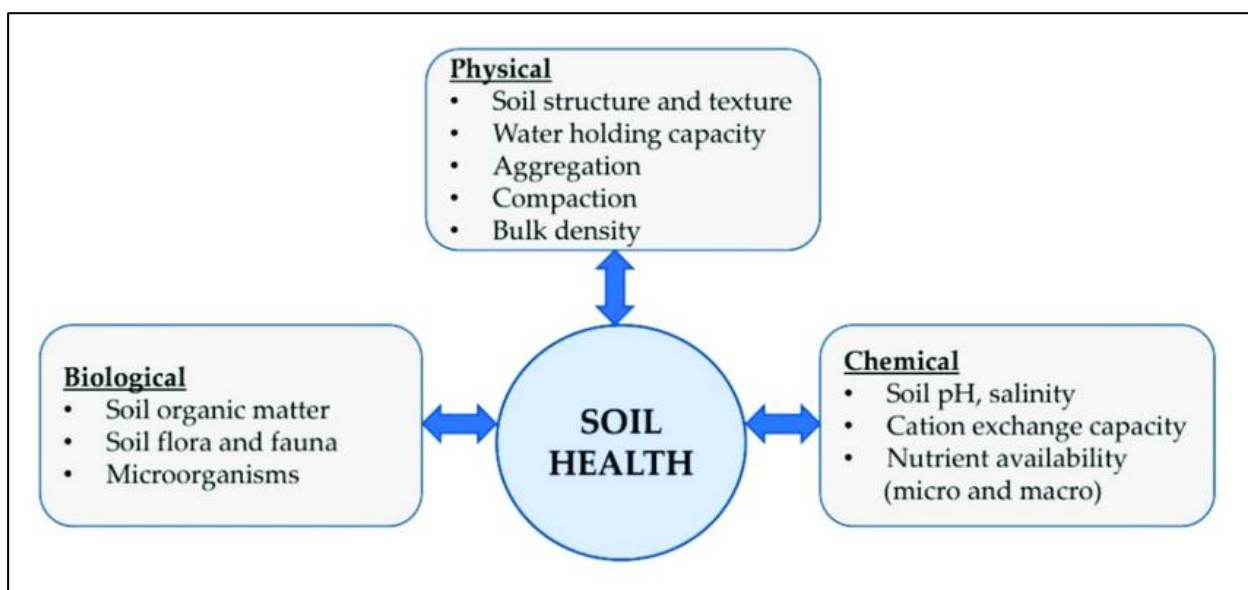


Figure 2.4.1: Representation of soil health characteristics (Thapa et al. 2021)

2.4.1. Physical indicators

Physical soil health indicators often used in agriculture are soil texture, soil structure, aggregate stability, bulk density, and soil water dynamics (porosity, permeability, and plant available water) (Bünemann et al. 2018; Muñoz-Rojas, 2018). Soil texture is the relative proportions of sand, silt, and clay (Brady & Weil, 2017) and is unlikely to be changed by agricultural activities (Zaaboubi et al. 2020). Soil textural classes are defined by the percentages (g/100 g mineral soil) of sand, silt, and clay, as indicated by the heavy boundary lines on the textural triangle (Figure 2.4.2) (Brady & Weil, 2017).

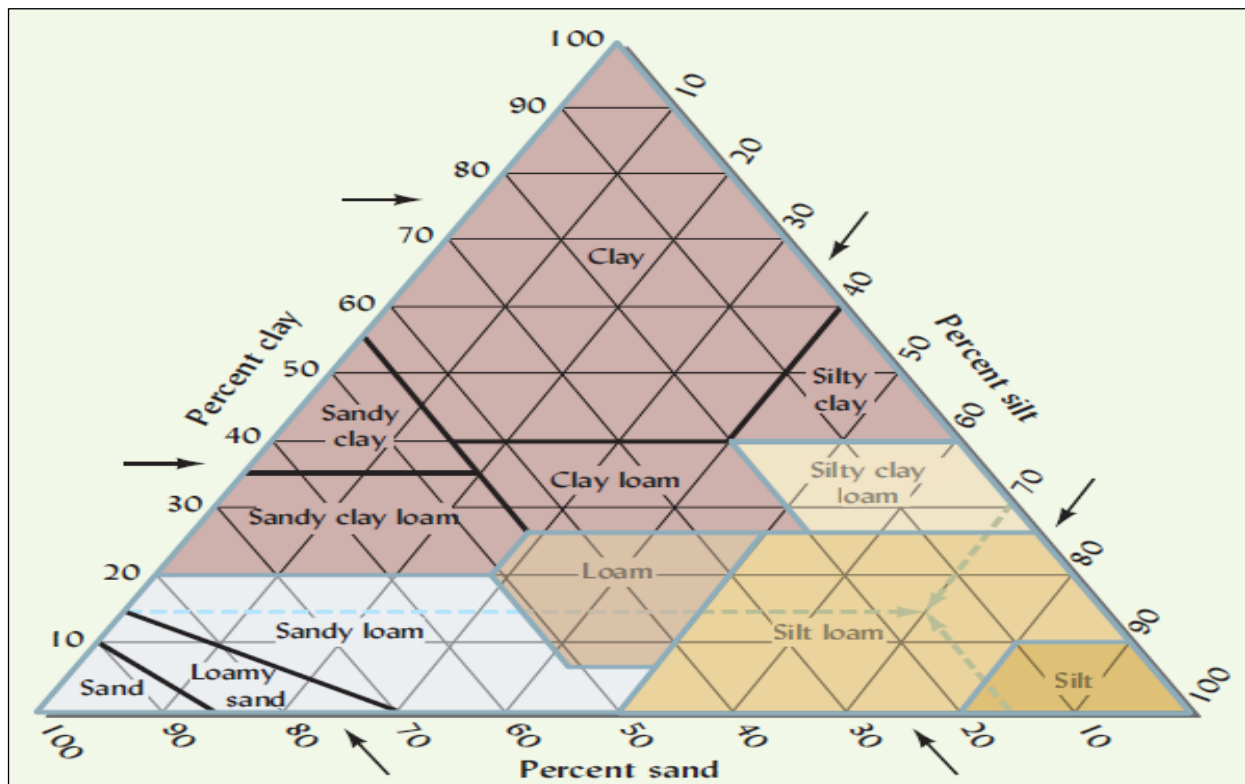


Figure 2.4.2: Soil textural classes represented on the textural triangle (Brady and Weil, 2017).

The arrangement of primary soil particles (sand, silt, and clay) into secondary particles (often referred to as peds) is referred to as soil structure (Brady & Weil, 2017). The difference in soil structure is classified based on size, shape, and degree of distinctness. Together with SOM, soil texture and soil structure coincide to form a stable aggregate environment that influences other physical soil health characteristics (Verhulst et al. 2010). The increase in clay content in soils is directly linked to higher levels of organic matter because clay particles bind organic material, protecting it from decomposition. Additionally, clay soils retain more moisture and nutrients, promoting greater plant biomass production, which contributes more organic residues to the soil. This enhanced protection and accumulation of organic material result in higher organic matter content in clay-rich soils (Brady & Weil, 2017).

A physical soil health characteristic that is often influenced by a stable aggregate environment in clay-rich soils is bulk density. Soil bulk density is “the mass of a unit volume of dry soil” (Brady & Weil, 2017). Maintaining good bulk density is important, as it sustains plant growth structural support, water, nutrient, and microbial movement, as well as soil aeration (Brady & Weil, 2017). The measurement of aggregate stability that contributes to good soil bulk density has been quantified as the portion of aggregates that remain after being exposed to destabilising stressors.

This serves as a valuable soil health indicator linked to soil hydrological functions, empirically to reduced erodibility, as well as root development and seed emergence (Rieke et al. 2022).

2.4.2. Chemical indicators

Among many chemical soil health indicators, it has been proposed that macro nutrient availability (Nitrogen (N), phosphorus (P) and potassium (K), and soil pH are often most relevant to the production of crops (Bünemann et al. 2018). Nitrogen, P, and K nutrient availability are essential to soil health as they form part of soil fertility (Bekele & Birhan, 2021; Brady & Weil, 2017; Jones, 2012; Nguemezi et al. 2020). Macronutrients are available in different quantities depending on the characteristics of the parent material of the soil. It is usually portrayed either as a percentage or in grams per kilogram (g/kg) of plant dry matter. The macronutrients are commonly absorbed in other forms that differ from their periodical appearance (Bekele & Birhan, 2021). A summary of representative nutrient quantities that could be found in plant dry matter, including the desired plant uptake forms, are presented in Table 2.4.1.

Table 2.4.1: Illustration of the amount and form of macronutrients in soil (Jones, 2012)

Nutrient	%	g/kg	Available form
Nitrogen (N)	1.50 – 6.00	15 – 60	NO ₃ ⁻ ; NH ₄ ⁺
Phosphorus (P)	0.15 – 1.00	1.5 – 10	H ₂ PO ₄ ⁻ ; HPO ₄ ²⁻
Potassium (K)	1.00 – 5.00	10 – 50	K ⁺
Calcium (Ca)	0.20 – 5.00	2.0 – 50	Ca ²⁺
Magnesium (Mg)	0.15 – 1.00	1.5 – 10	Mg ²⁺
Sulphur (S)	0.15 – 0.50	1.5 – 5.0	SO ₄ ²⁻

Nitrogen has a vital role in the vegetative phase of crop production and is responsible for the synthesis of starch in leaves, protein synthesis, and the production of amino acids (Jones, 2012; Kumar et al. 2021). Phosphorus is a primary component of nucleic acids, plant cell membranes, and enzyme activity. It aids in various processes, such as photosynthesis, carbohydrate (C_xH₂O)_y metabolism, and energy production (Kumar et al. 2021). Potassium is required in the maintenance of plant water status, turgor pressure, and is involved in the regulation of its stomata. Soil pH influences plant growth, soil structure, nutrient availability, microbial activity (including many other soil chemical reactions), and soil health. Neina (2019) reports that pH fluctuations may occur as a result of 1) the leaching of primary cations that leave soils with excess amounts of H⁺ and Al³⁺

2) the dissolution of CO_2 found in soil water, leading to the formation of carbonic acid and release of H^+ ions 3) nitrification of NH_4^+ to NO_3^- that produces H^+ ions.

2.4.3. Biological indicators

Haney et al. (2018) stress the importance that soil health includes diverse soil organism communities with beneficial symbiotic associations such as plant root development, essential nutrient cycling, soil structure improvements, and ultimately improved crop production. Building on this statement Haney et al. (2018) highlight that in previous years, the soil's biological components have been greatly neglected because producers often neglect soil as a biological living system. This observation is supported by a study conducted by Bünemann et al. (2018), where 40% of the available data correlating to soil health made no mention of any biological indicators. From the rest of the data, Bünemann et al. (2018) conclude that the most important biological indicators are soil organic matter content, microbial biomass, N mineralisation, and soil respiration.

Soil organic matter can be seen as a mixture of biogenic components of different proportions and stages, decomposed plant materials, and several microorganisms (Arias et al. 2005). The quantity of organic matter in most soils is relatively small but profoundly influences all soil functions (Brady & Weil, 2017). The total soil organic matter pool is often too large to detect and manage, therefore qualitative information on soil organic matter provides more information and is often used as the actual biological indicator of soil health (Bünemann et al. 2018). One of these qualitative gauges are soil respiration, which is the biological oxidation of soil organic matter to CO_2 through microorganisms (Arias et al. 2005). The most abundant microorganism are free-living soil bacteria known to promote plant growth and are referred to as plant growth-promoting rhizobacteria (PGPR) (Hayat et al. 2010). Plant growth-promoting rhizobacteria are also capable of transformation, mobilization, and solubilization of nutrients that are essential to plant growth (Hayat et al. 2010). Soil organic carbon (SOC) is another qualitative gauge, which is the measurable amount of carbon present in SOM and consists of plant, as well as animal remains at different decomposition periods, including microbial biomass with their by-products (Lal, 2016). The amount, quality, and turnover of soil organic carbon is vital to soil health because it influences soil structure and aggregation, soil water retention, nutrient retention, nutrient transformations, and gaseous emissions (Lal, 2016).

2.5. SOIL HEALTH ASSESSMENT AND SCORING

Soil health is evaluated through physical, chemical, and biological indicators that relate to specific soil functions (e.g. soil as a supporter of plant growth) (Haney et al. 2018; Lal, 2016). Bünemann et al. (2018) discuss the difference between analytical and visual approaches to soil health assessments. The visual assessments provide easy soil health identification based on characteristics (e.g. plant growth) but often require additional analytical assessments to be substantial. By keeping the focus on analytical soil health identification, Table 2.5.1 summarises the primary analytical frameworks in different geographic locations (Bünemann et al. 2018).

Table 2.5.1: Soil quality Assessment frameworks developed in different regions (Bünemann et al. 2018; Haney et al. 2018).

Location	Analytical framework name	Measurement Objective
Canada	National soil quality monitoring program	Assess the status and trends of soil health
USA	Soil management assessment framework (SMAF)	Assess inherent soil quality and susceptibility to change, evaluate management practices, and educate about soil quality.
	Cornell Soil Health Test (CASH)	Assess soil health, address soil degradation, and increase productivity.
Australia	Soil Quality Website	Benchmark sites, soil quality monitoring and education
New Zealand	Soil <u>I</u> ndicator Assessment (Sindi)	Assess soil quality for environmental reporting
France	Observatoire de la Qualité des Sols (OQS), Réseau des mesures de la qualité des sols (RMQS)	Assess soil quality for environmental protection, food security and sustainable management practices
Ireland	Soil quality assessment research project (SQARE)	Assessment of soil functions
The Netherlands	National Soil Quality Monitoring Network	Assess soil quality and land-use effects

EU	European Soil Monitoring and Assessment Framework	Provide objective, reliable, and comparable information at the European level
----	---	---

Analytical identification was done in this study using the Haney Analysis. This tool is a relatively new soil health assessment framework that also evaluated the soil as a living ecosystem and not just as physio-chemical components (Haney et al. 2018).

2.5.1. Haney Analysis and Soil Health Score

The Haney Analysis has been developed through the integration of mainly biological and chemical soil properties. Making use of methods commonly used in commercial soil testing laboratories. The following list represents the biological and chemical parameters included in the Haney Soil Health analysis.

a) Biological parameters

- Active Carbon (mg/kg)
- Organic matter %
- Soil respiration (CO²-C)
- H₂O Total organic C (ppm)
- Soil Organic Carbon %
- Water Extractable Organic C % of Tot SOC
- % Microbially Active Carbon
- Organic C:N (ratio)

- Organic N Release (ppm)
- Organic N Reserve (ppm)
- Total available N (ppm)
- N kg/ha
- H3A Total Phosphorus (ppm)
- H3A Inorganic Phosphorus (ppm)
- H3A Organic Phosphorus (ppm)
- Total available Phosphorous (ppm)
- P kg/ha
- P Organic:Inorganic Organic P Release (ppm)

b) Chemical indicators

- Soil pH
- H₂O Total N (ppm)
- H₂O Organic N (ppm)
- H3A Nitrate (ppm)
- H3A Ammonium (ppm)
- H3A Inorganic Nitrogen (ppm)
- Total N (Organic + Inorganic) (ppm)
- Organic N: Inorganic N ratio

- Organic P Reserve (ppm)
- H3A Potassium (ppm)
- K kg/ha
- H3A Calcium (ppm)
- H3A Aluminum (ppm)
- H3A Iron (ppm)
- H3A Sulfur (ppm)
- H3A Zinc (ppm)
- H3A Manganese (ppm)

- H3A Copper (ppm)
- H3A Magnesium (ppm)
- H3A Sodium (ppm)
- % P Saturation (Al/Fe)
- % P Saturation (Ca)
- Calcium: Magnesium
- Calcium: Magnesium/ Aluminium
- Magnesium: Potassium
- Potassium: Sodium
- Calcium Ca %
- Magnesium Mg %
- Potassium K %
- Sodium Na

The Haney analysis joins multiple parameters to present quantities of plant available N, P, and K, and provide an indication of soil health as related to nutrient and C cycling. Compared to that of traditional tests that only estimate one form of plant available N. Traditional tests also simplify soil C, where the Haney Analysis integrates water-extractable organic C (WEOC) that reflects the quality of soil organic C. The Haney Analysis also uses WEO C:N ratios as a more sensitive indicator of the active soil pools. H3A extractant (which is a weak organic acid mimicking plant root exudates) is used to measure plant-available P (Haney et al. 2018).

Many CO₂ respiration tests are done in a steady state (constant temperature, oxygen, and water content), which differs from the Haney Analysis that determines CO₂ released by soil microorganisms, one day (24 hours) after the soil has been dried and rewetted. This mimics the in-field conditions of rain and irrigation. The laboratory that completes the Haney Analysis use the results to generate a soil health score. Calculation of the soil health score (SHS), is done by using Equation 2.5.1. Water extractable organic C (WEOC) used in the soil health score calculation, reflect the quality of SOC as an energy provider for microbial activity. Water extractable organic N (WEON) is the measurable amount organic N available to plant uptake and crucial in the production of crops (Haney et al. 2018).

Equation 2.5.1: Equation used to calculate the soil health score

$$SHS = \left(\frac{1 \text{ Day } CO_2 C}{10} \right) \times \left(\frac{WEOC}{100} \right) \times \left(\frac{WEON}{10} \right)$$

The intent of the Haney Analysis was to bring forward an analysis framework (tool) that would help laboratories and researchers include microbiological soil nutrient cycling. By doing so, the Haney Analysis is aimed at increasing the efficiency of fertiliser use by exposing plant available nutrients (Haney et al. 2018).

2.5.2. Best subset (proxy) indicators

The advancement of soil health monitoring has led to comprehensive assessments that evaluate a range of physical, chemical, and biological indicators that measure soil health. While a broad understanding can provide valuable insights, Fine et al. (2017) highlight the importance of focusing on indicators most relevant to system processes for precise ecosystem monitoring, which often does not require an extensive framework. The pursuit of practicality, efficiency, and cost-effectiveness has long driven the search for singular indicators and specific subsets of indicators for soil monitoring (Doran et al. 1994). Singular indicators offer direct insights into specific soil properties, while subsets provide a more holistic understanding of soil health by integrating multiple parameters.

Fine et al. (2017) demonstrated that when selecting a single indicator to assess soil health, active carbon, organic matter, and soil respiration (in that order) are the most effective. For a two-indicator approach, penetration resistance and active carbon are ideal, representing a combination of biological and physical properties. Van Antwerp (2005) further emphasised that soil organic carbon, particularly its labile fraction, is the most influential single factor in determining soil health and quality due to its broad impact on chemical, physical, and biological properties. Adding that a minimum data set (MDS) for soil health assessments is shaped by considerations of practicality and cost-effectiveness. Whereby the suggested list of soil health indicators from which a minimum set, for use in the South African sugar industry, would be: Physical parameters – texture, dispersion index, aggregate stability, plant-available water capacity, soil colour – Chemical parameters - FAS routine (soil), mineralisable C, mineralisable N, labile C, total C – Biological parameters - Microbial biomass C, microbial biomass N, basal respiration, dehydrogenase activity, bacteria to fungal ratio, nematodes.

2.6. IMPORTANCE OF ECOTOPES AS A SPATIAL UNIT

Ecotopes were originally defined as the total environmental niche of a species, distinct yet related to its habitat (Whittaker et al. 1973). More recently, Hong et al. (2004) described an ecotope as a distinct ecological unit incorporating various landscape elements, including topography, vegetation, climate, and soil conditions. It is the smallest defined area used to characterise and identify a landscape and serves as a fundamental spatial unit for ecosystem evaluation, conservation, and management. Hensley et al. (2019) further elaborates that an ecotope represents a specific class of land within a larger ecosystem or biome, distinguished by its unique physical, chemical, and biological properties. This classification may also include, where

necessary, detailed factors such as soil characteristics (e.g., slope, aspect, and soil type) and climatic information (e.g., temperature and precipitation) to define the smallest unit of such land.

In South Africa, the larger ecosystems from which ecotopes are derived from is classified within the biome regions of South Africa. Palmer et al. (2005) state that these biome regions are Grasslands, Savannah, Succulent Karoo, Nama Karoo, Forest, Fynbos, Desert, and Thicket (Figure 2.6.1).

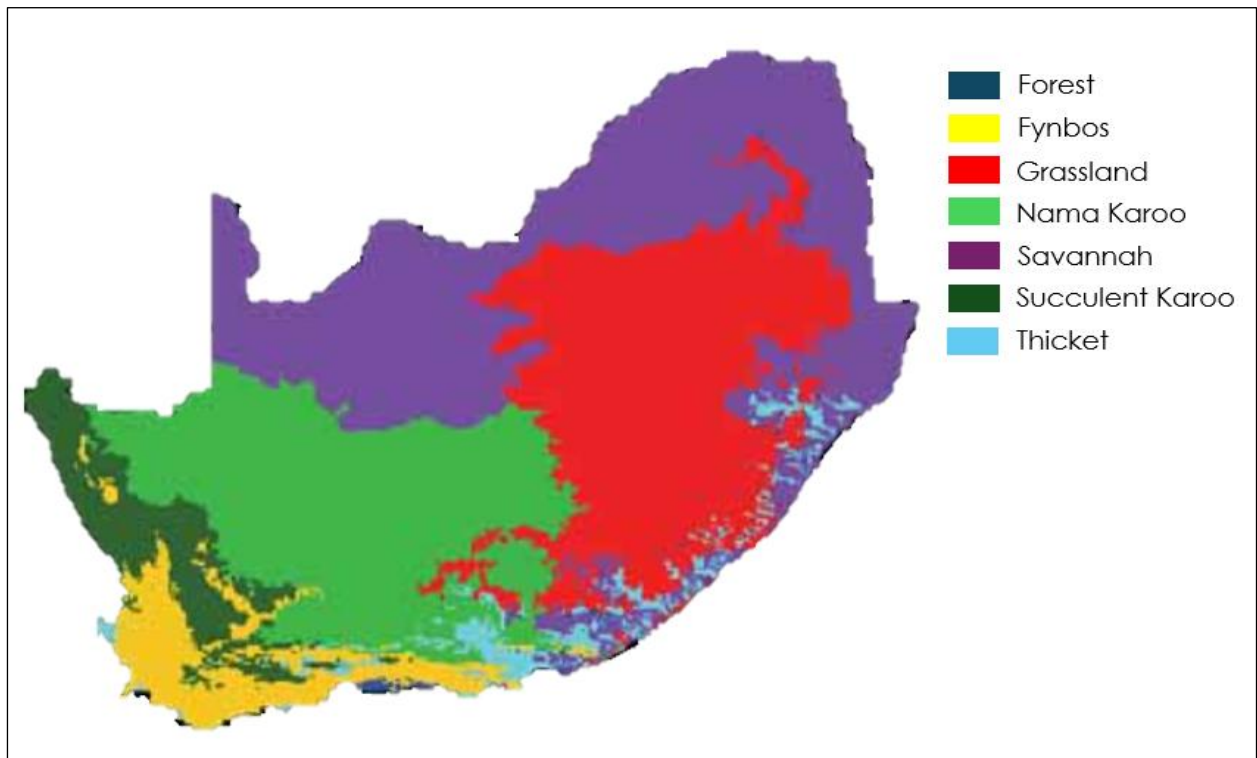


Figure 2.6.1: Different biome regions of South Africa (Palmer et al. 2005)

Each biome is defined by unique abiotic and biotic features, which can be studied on a refined scale to identify and distinguish ecotopes. For example, analysing factors such as climate (primarily temperature and precipitation), organisms (e.g., vegetation and human activity), topography, and parent material within a specific area highlights variations in inherent soil formation processes (Brady & Weil, 2017). These variations can be used to classify and differentiate areas as distinct ecotopes. Each ecotope will have its own unique soil formation, characterised by inherent physical, chemical, and biological properties.

2.7. LITERATURE CONCLUSION

The literature review has comprehensively examined the evolution of agricultural practices in South Africa, from conventional methods to the adoption of conservation and regenerative agriculture. While conventional agriculture has significantly advanced crop production, it has also contributed to severe environmental degradation, including soil erosion, biodiversity loss, and water pollution. These challenges highlight the urgent need for sustainable agricultural practices that not only maintain productivity but also restore ecological balance. Soil health is a central factor in conservation and regenerative agriculture, underpinning ecosystem functionality, crop productivity, and long-term sustainability. Conservation agriculture, with its core principles of minimal soil disturbance, crop rotation, and permanent soil cover, has been widely recognised for improving soil structure, enhancing microbial activity, reducing erosion, and increasing water retention. Regenerative agriculture builds upon these principles by incorporating holistic approaches such as cover cropping and livestock integration, aiming to actively restore and enhance soil ecosystems. However, the effectiveness of these practices depends on the ability to monitor soil health accurately and systematically. Soil health assessment frameworks, such as the Haney Analysis, have emerged as essential tools for evaluating the biological, chemical, and physical properties of soil. These frameworks integrate multiple indicators to provide a comprehensive understanding of soil functionality, including nutrient cycling, microbial activity, and organic matter content. Regular soil health monitoring enables farmers to make informed management decisions, ensuring that conservation and regenerative practices achieve their intended benefits. Despite the comprehensive nature of such frameworks, there is growing interest in identifying singular proxy indicators that can serve as efficient and cost-effective measures of soil health. Certain indicators, such as soil respiration, aggregate stability, or enzyme activity, may provide valuable insights into soil conditions without requiring extensive testing. The development of reliable proxy indicators could enhance large-scale soil monitoring efforts, making sustainable agriculture more accessible to resource-limited farmers while supporting broader adoption of conservation and regenerative practices. This review sets the foundation for understanding the critical role of soil health in mitigating environmental degradation and ensuring the viability of farming systems in South Africa. Future research and policy development should prioritise the refinement of soil health monitoring frameworks, the identification of effective singular indicators, and the scaling of sustainable agricultural practices to promote long-term agricultural resilience, economic stability, and food security.

CHAPTER 3 METHODOLOGY

3.1. TRIAL SITES

Under leadership of Asset Research with funding from the Maize Trust (South Africa), a decision was made to undertake research and develop conservation agriculture systems in collaboration with local producers. This establishment led to the launch of a Farmer Initiative Programme (FIP), which included participation from the following trial sites: Nicol de Vos (NV) (Kinross, Mpumalanga), managed by Nicol de Vos & Sons; Hendrik Odendaal (HO) (between Villiers and Standerton, Mpumalanga), managed by Hendrik Odendaal; and Nant Yzel (NY) (Marquard, Maluti), managed by Yzel Farms. In Table 3.1.1 the trial site coordinates are provided with Figure 3.1.1 representing a visual representation.

Table 3.1.1: Location details of the trial sites, including the implementing farmer, the nearest town, and the coordinates of each trial.

Farmer	Closest town	Coordinates
Nicol de Vos	Kinross	26°21'38.56" S 29°11'47.02"E
Hendrik Odendaal	Villiers – Standerton	26°57'23.19"S 28°57'30.31"E
Nant Yzel	Marquard	28°42'24.93"S 27°23'11.00"E



Figure 3.1.1: Locality map of the three trial sites that represent the ecotopes in this study (Own Compilation)

These three distinct trial sites were evaluated to determine characteristics of the ecotope. Whereby ecotopes are defined as distinct ecological units that encompass various landscape elements, including topography, vegetation, climate, and soil conditions. They represent the smallest defined area used to characterise and identify a landscape, serving as fundamental spatial units for ecosystem evaluation, conservation, and management (Hong et al. 2004).

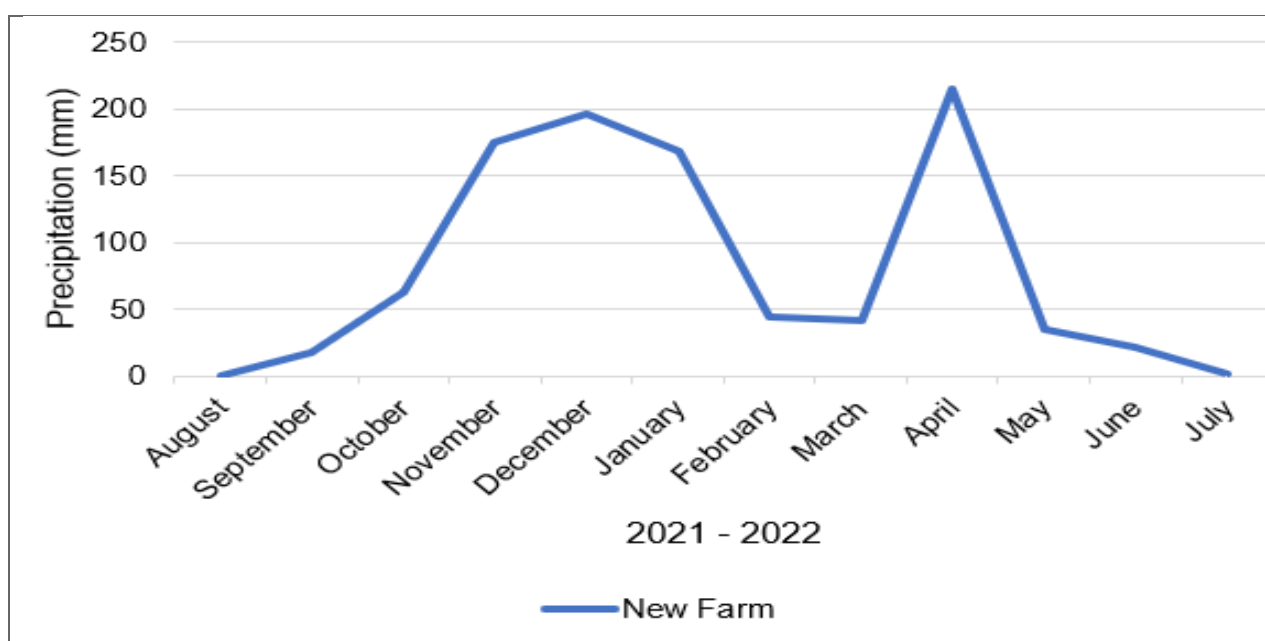
It is important to note that each of the three ecotopes was characterised based on its soil properties and climatic conditions (Hensley et al. 2019). Ideally, long-term averages of rainfall and temperature would have provided a more comprehensive distinction between ecotopes. However, due to limited historical climatic data, an alternative approach was taken. To establish site-specific ecotope characteristics accurately, precipitation was monitored from August 2021 to July 2022, alongside detailed recording of edaphic (soil-related) conditions. This allowed for a real-time assessment of climate-soil interactions at each site, ensuring that the classification of ecotopes was based on the most relevant and directly observed data. Although additional trial sites were monitored, the sites included in this study were selected as the most representative in line with the study's objectives.

3.1.1. Nicol de Vos

It was noted that precipitation at this ecotope is higher during the initial growth period in the first summer phase, with even distribution between November and January. This is followed by a plateau in February and March, after which precipitation will gradually decline. A sharp increase in late summer (April) will precede significantly lower rainfall during the winter months, starting in May. The average precipitation between August 2021 and July 2022 at Nicol de Vos was 88 mm. Table 3.1.2 presents this information in relation to the ecotope identification (Nicol de Vos).

Table 3.1.2: Precipitation information recorded to establish the Nicol de Vos ecotope (Knot et al. 2022)

Ecotope	Events	Aug 21	Sept 21	Oct 21	Nov 21	Dec 21	Jan 22	Feb 22	Mar 22	April 22	May 22	Jun 22	Jul 22
Nicol de Vos	77	-	18	63	175	196	168	44	41	215	35	22	1



The predominant soils found at Nicol de Vos was Bainsvlei, characteristic of Orthic-A over a soft Plinthic-B with a red Apedal-B. Table 3.1.3 summarises the information gathered on-site to distinguish the ecotope.

Table 3.1.3: The edaphic information recorded to establish the Nicol de Vos ecotope (Knot et al. 2022)

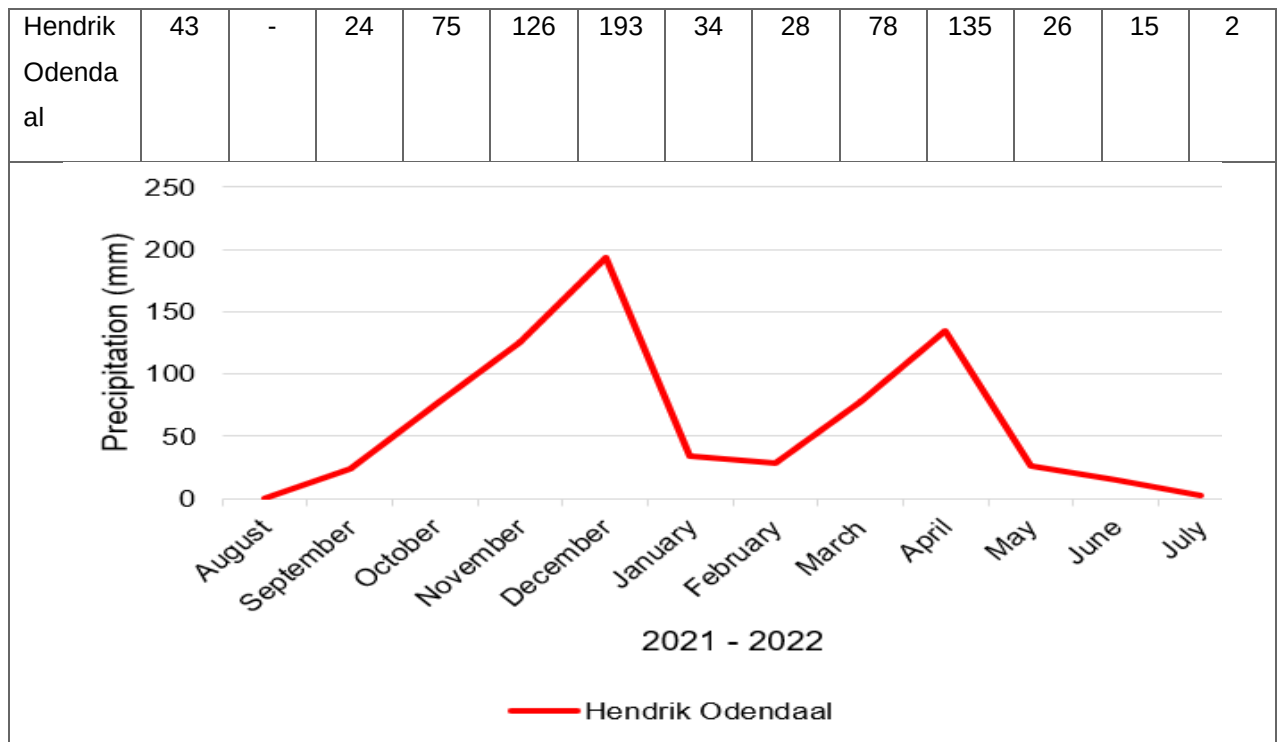
Trial	Coordinates	Soil type	Soil depth	Soil structure
Nicol de Vos (Nicol de Vos & Sons)	26°21'38.56" S 29°11'47.02"E	Bainsvlei - to a lesser extend Oackly, Valsrivier and Bloemdal	1200 cm	Sandy, 11-15% clay

3.1.2. Hendrik Odendaal

It was noted that precipitation at this ecotope is less evenly distributed during the initial growth period. Unlike the Nicol de Vos ecotope, precipitation peaks in December, followed by a sharp decline in January, resulting in an earlier plateau between January and February. The average precipitation between August 2021 and July 2022 at Hendrik Odendaal was 66mm. Table 3.1.4 presents the recorded data used to define the Hendrik Odendaal ecotope.

Table 3.1.4: Precipitation information recorded to establish the Hendrik Odendaal ecotope (Knot et al. 2022)

Ecotope	Events	Aug 21	Sept 21	Oct 21	Nov 21	Dec 21	Jan 22	Feb 22	Mar 22	April 22	May 22	Jun 22	Jul 22



Sepane was found at Hendrik Odendaal and are slightly deviant from the other trial sites, it is characteristic of a Orthic-A, Pedocutanic-B, and Prismaeutanic-C. This type of soil has much more clay content than the other two ecotopes. Table 3.1.5 summarises the information gathered on-site to distinguish the ecotope.

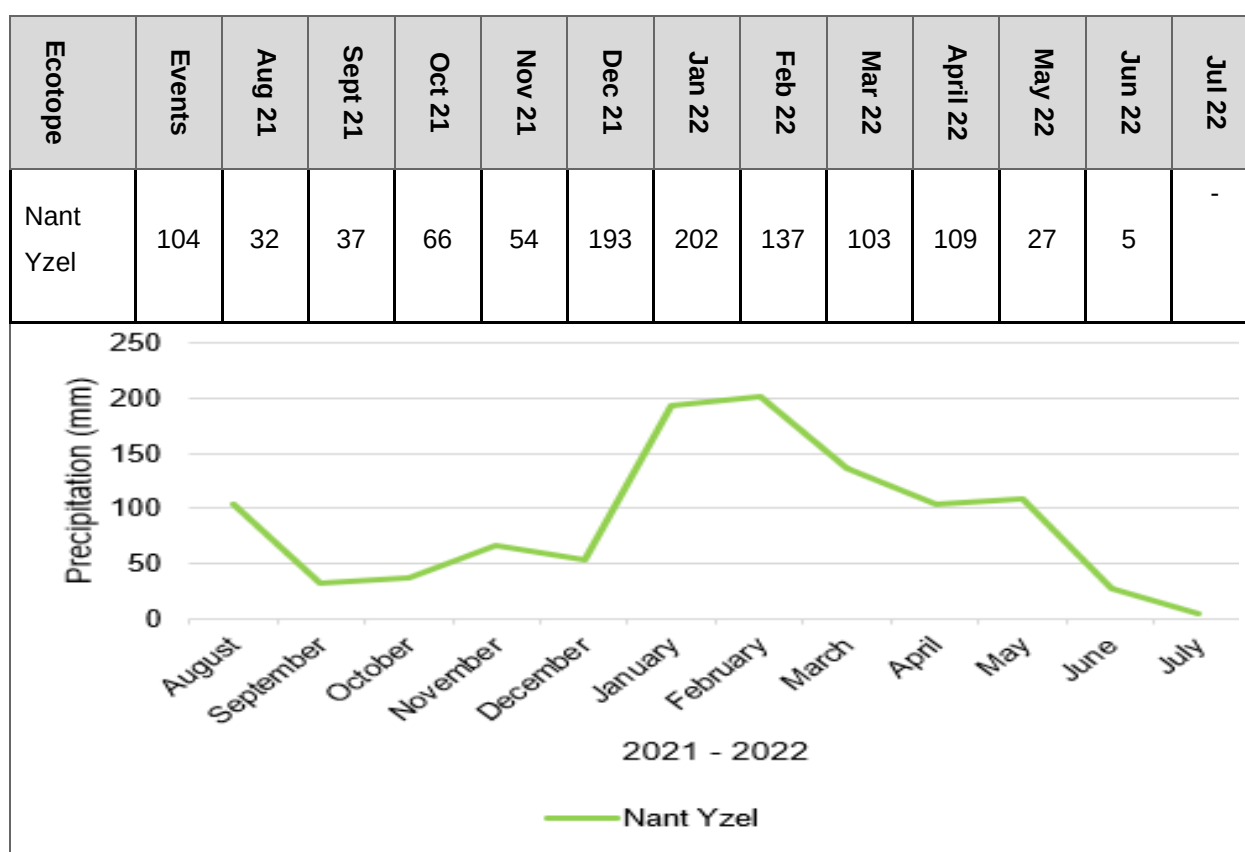
Table 3.1.5: The edaphic information recorded to establish the Hendrik Odendaal ecotope (Knot et al. 2022)

Trial	Coordinates	Soil type	Soil depth	Soil structure
Standerton (Hendrik Odendaal)	26°57'23.19"S 28°57'30.31"E	Sepane	800-900 cm	40-47% clay

3.1.3. Nant Yzel

At Nant Yzel, there was not much precipitation during the initial growth period, which is a notable difference between this ecotope, Nicol de Vos, and Hendrik Odendaal. From January, Nant Yzel receives good precipitation that gradually declines until April. This also differs from the other ecotopes, as Nant Yzel receives the precipitation spike in May. The average precipitation between August 2021 and July 2022 at Nant Yzel was 87 mm. Table 3.1.6 shows the information recorded to define the ecotope (Hendrik Odendaal).

Table 3.1.6: Precipitation information recorded to establish the Nant Yzel ecotope (Knot et al. 2022)



Nant Yzel has Avalon and Westleigh soils, which are similar in characteristics and are defined by Orthic-A over a soft Plinthic-B. The soils here have the least clay content. Table 3.1.7 summarises the information gathered on-site to distinguish the ecotope.

Table 3.1.7: The edaphic information recorded to establish the Nant Yzel ecotope (Knot et al. 2022)

Trial	Coordinates	Soil type	Soil depth	Soil structure
Marquard (Yzel Farms)	28°42'24.93"S 27°23'11.00"E	Avalon (North) Westleigh (South)	610 cm 570 cm	Sandy soil with 7% clay

3.2. TRIAL DESIGN

At the participatory planning event, it was agreed that the conventional agriculture (Conv) systems at both HO and NV would feature maize–soy rotations each cropping season to maintain economic viability. This implementation was consistent across seasons, with the same agricultural strips used for the same crops throughout the study. At NY, the Conv systems were limited to maize production each season, with no variation in implementation. These setups were designed to ensure the practice of monoculture cropping closely associated with conventional

agriculture. The conservation agriculture (Cons) systems followed maize–soy rotations each season, alternating crops on each designated agricultural strip over the study period. The regenerative agriculture (Regen) systems had four rotations represented at any given time, each starting with a different initial crop: maize – winter cover crops, summer cover crops – winter cover crops, soy – winter cover crops, and summer cover crops – winter cover crops. This setup meant one treatment began with maize, one with soy, and two with summer cover crops. By the end of this study period each initial crop shifted to start on a different agriculture strip. Table 3.2.1 and Table 3.2.2 shows the layout of the trials as originally planned out.

Table 3.2.1: Original layout of the trials set out in Mpumalanga (Hendrik Odendaal and Nicol de Vos) (HO and NV): summer cover crops (SCC), winter cover crops (WCC)

Production year	Regenerative agriculture				Conventional agriculture		Conservation agriculture	
2020-2021	Maize - WCC	SCC - WCC	Soy - WCC	SCC - WCC	Maize	Soy	Maize	Soy
2021-2022	SCC - WCC	Soy - WCC	SCC - WCC	Maize - WCC	Maize	Soy	Soy	Maize
2022-2023	Soy - WCC	SCC - WCC	Maize - WCC	SCC - WCC	Maize	Soy	Maize	Soy

Table 3.2.2: Original layout of the trials set out in Maluti (Nant Yzel) (NY): summer cover crops (SCC), winter cover crops (WCC)

Production year	Regenerative agriculture				Conventional agriculture	Conservation agriculture	
2020-2021	Maize - WCC	SCC - WCC	Soy - WCC	SCC - WCC	Maize	Maize	Soy
2021-2022	SCC - WCC	Soy - WCC	SCC - WCC	Maize - WCC	Maize	Soy	Maize
2022-2023	Soy - WCC	SCC - WCC	Maize - WCC	SCC - WCC	Maize	Maize	Soy

Each farming system was replicated three times per cropping season at all three ecotopes. This meant that the Conv systems at HO and NV had six (6) dedicated strips each for the monoculture production of maize and soy, while NY had three (3) strips for maize only. The Cons system across all ecotopes included six (6) strips each for rotating maize and soy. The Regen system at

each ecotope had twelve dedicated strips. In total, NV had twenty-seven (27) strips throughout all cropping seasons, which included three (3) uncultivated areas serving as the Uncult system. Hendrik Odendaal followed the same allocation. At NY, however, there were twenty-four (24) strips due to the continuous maize monoculture in the Conv system, without the inclusion of soy as implemented at NV and HO.

Maize implementation was consistent across HO, NV, and NY during the first two cropping seasons, with the same cultivar used across all systems within each trial. In the third season, HO selected a different cultivar and slightly increased the plant population, while NV and NY maintained consistency. For soy, implementation remained consistent across HO, NV, and NY during the first two cropping seasons, again using the same cultivar within each trial. However, in the final season, all three ecotopes adopted a different cultivar and varied planting quantities (Table 3.2.3).

Table 3.2.3: Cultivar and plant population information for maize and soy production for each ecotope during three consecutive cropping seasons

Cropping season	Crop	Ecotope	Cultivar	Plant population ha⁻¹
Cropping season 1 (2020 – 2021)	Maize	Nant Yzel	DKC7276BR	50,000
	Maize	Nicol de Vos	P2432R	50,000
	Maize	Hendrik Odendaal	PIONEER P2137BR	42,000
Cropping season 2 (2021 – 2022)	Maize	Nant Yzel	DKC7276BR	50,000
	Maize	Nicol de Vos	P2432R	50,000
	Maize	Hendrik Odendaal	PIONEER P2137BR	42,000
Cropping season 3 (2022 – 2023)	Maize	Nant Yzel	DKC74-26R	50,000
	Maize	Nicol de Vos	P2432R	50,000
	Maize	Hendrik Odendaal	DKC64-52R	45,000
Cropping season	Crop	Ecotope	Cultivar	Plant population ha⁻¹
Cropping season 1 (2020 – 2021)	Soy	Nant Yzel	PAN 1623R	250,000
	Soy	Nicol de Vos	PHI 48T48R	300,000
	Soy	Hendrik Odendaal	Don Mario 5302R	300,000

Cropping season 2 (2021 – 2022)	Soy	Nant Yzel	PAN 1623R	250,000
	Soy	Nicol de Vos	PHI 48T48R	300,000
	Soy	Hendrik Odendaal	Don Mario 5302R	300,000
Cropping season 3 (2022 – 2023)	Soy	Nant Yzel	PAN1623	350,000
	Soy	Nicol de Vos	PAN1555R	280,000
	Soy	Hendrik Odendaal	DM5953	330,000

Summer cover crops were implemented using a specific mixture per hectare. Table 3.2.4 details the components and application rate of this summer cover crop mixture. The implementation remained consistent across all cropping seasons at each of the three ecotopes, with no changes from one season to the next.

Table 3.2.4: Consistent summer cover crop mixture as per components and application rates per hectare for each ecotope

Trial area	Seed description	Quantity applied per hectare (kg)
Nant Yzel	- Berseem Clover	2
	- Bladrammanas (leaf radish)	2
	- GV	8
	- Common vetch	2
	- Oats	20
	- Tiller radish	1
	- Rye	5
	- Winter forage (Austrian) pea	5
Nicol de Vos	- Baster babala	2
	- Babala	10
	- Cowpea	10
	- Sunn hemp	8
	- Tiller radish	1
Hendrik Odendaal	- Babala	6
	- Fodder sorghum	6
	- Japanese millet	2
	- Saia oats	4
	- Sunn hemp	4
	- Dolichos	3
	- Flax	2
	- Buck wheat	2
	- Sunflower	1
	- Fodder radish	0.3
	- Tiller radish	0.5

Farmers used a mixture of winter cover crops to follow summer cover crops (after termination by grazing or mimicked grazing/chemical burndown), which typically consist of Triticale, Saia oats,

Wizard oats, and Tiller radish. Application time differed throughout the study as variations in rainfall and temperature occurred. The application of only wheat and rye as winter cover crop mixtures for farmers across all ecotopes were common because temperatures become too low for other considerations. Implementation was consistently aimed to be in August. Oats and Rye (with occasional inclusion of vetch and radish) were the implementation options following the production of soya.

Since the same crop strain and quantity were consistently implemented across all three ecotopes during the three cropping seasons, the primary focus was on establishing the different farming systems. Each farming system varied in implementation according to the principles of Conv, Cons, and Regen practices.

3.2.1. Conventional farming systems implementation information

In the Conv farming systems, each ecotope experienced mechanical soil disturbance prior to planting, using chisel ploughing/ripping and disking to prepare the soil for consecutive maize at NY, and for maize in the summer and soy in the winter at HO and NV. Additionally, when soil preparation occurred well in advance of planting, a chemical burndown in the form of herbicide was applied. Further herbicide applications were made at planting and as needed throughout the cropping season, as detailed in (Annexure A).

It was agreed that only the maize crops at each ecotope would receive fertiliser applications during each cropping season under the Conv farming system. The soy crops within the Conv systems did not receive any fertiliser. It should also be noted that only the N:P:K ratio was recorded, although the fertilisers used contained additional macronutrients. (Table 3.2.5) serves as a summary of total fertiliser application.

Table 3.2.5: Conventional farming system total fertiliser application kg/ha⁻¹ (N:P:K) for Nicol de Vos (NV), Hendrik Odendaal (HO), and Nant Yzel (NY) during each cropping season for the production of maize

Ecotope	Cropping season 1 (2020-2021)			Cropping season 2 (2021-2022)			Cropping season 3 (2022-2023)		
	N	P	K	N	P	K	N	P	K
	2020/2021			2021/2022			2022/2023		
NV	93	33	44	77	32	24	129	28	32
HO	77	22	12	73	12	12	88	22	13
NY	60	9	4	38	10	4	48	12	12

3.2.1. Conservation farming systems production information

The Cons systems were characterised by the absence of any mechanical soil disturbance in preparing for maize or soy planting. Instead, only a chemical burndown through herbicide application was used prior to planting and continued as needed during the cropping season (Annexure B). This approach marked the primary difference between the Conv and Cons systems regarding herbicide application.

As for the application of fertiliser under the implementation of the Cons system, only the maize crops at all three ecotopes received fertiliser application, which means that the soy in the rotation did not receive any fertiliser application. It should be noted that the fertiliser application for Cons systems was exactly the same as for the Conv systems.

3.2.1. Regenerative farming systems production information

In the Regen farming systems, no mechanical disturbances were involved in the planting of maize or soy, and chemical burndown practices were the same as those used in the Cons systems. The main distinction between Regen and Cons systems was the integration of cover crops. In this approach, summer cover crops (SCC) were planted during the summer cash crop season with chemical burndown preparation applied (Annexure C). These SCC were then grazed either in preparation for winter cover crops (WCC) or grazed and chemically burned down in preparation for planting maize or soy. When WCC followed either maize or soy, the cash crop was harvested, and WCC was implemented without any soil disturbance or herbicide application.

As for fertiliser application, only the maize crops as cash crop, at all three ecotopes received fertiliser application, which means that the soy in the rotation did not receive any fertiliser application. What differentiated fertiliser application at the Regen systems from the Conv and Cons systems, where that application was to reduce by $\pm 10\%$ each year. It should also be noted that from the cover crops, only the SCC received fertiliser application (Table 3.2.6).

Table 3.2.6: Regenerative farming system total fertiliser application kg/ha⁻¹ (N:P:K) for Nicol de Vos (NV), Hendrik Odendaal (HO), and Nant Yzel (NY) during each cropping season for the production of maize

Ecotope	Crop	Cropping season 1 (2020-2021)			Cropping season 2 (2021-2022)			Cropping season 3 (2022-2023)		
		N	P	K	N	P	K	N	P	K
		2020/2021			2021/2022			2022/2023		
NV	Maize	93	33	34	57	16	12	113	13	25
HO		77	22	12	72	10	12	76	12	7

NY		60	9	4	34	9	4	48	12	12
NV	SCC	56	11	14	15	11	14	17	13	17
HO		30	-	12	25	13	12	-	-	-
NY		-	-	-	-	-	-	23	-	-

Lambs were used during grazing to terminate summer cover crops at Nant Yzel. The lambs grazed during daytime and were kraaled at night. A similar technique was used at Hendrik Odendaal, where heifers grazed during the day and kept overnight at veld adjacent to the trial. Nicol de Vos was not grazed but slashed. Following a technique to mimic livestock grazing where manure was added in line with livestock digestion.

3.3. SOIL SAMPLING METHODOLOGY

Soil sampling was conducted across three consecutive cropping seasons (2020/2021, 2021/2022, and 2022/2023) in alignment with the trial design. All sampling occurred in February. One sample was collected from each dedicated strip within each farming system throughout the implementation. This resulted in a total of twenty-seven (27) samples being collected at NV and HO, respectively, and twenty-four (24) samples at NY for each cropping season. The first sampling point was located 100 metres from the start of the strip and was geolocated to ensure accurate sampling during subsequent replicates. As shown in Figure 3.3.1, each sample consisted of four sub-samples taken within a 1-metre radius around the central sample point, resulting in a total of five samples per location.



Figure 3.3.1: A) Soil sampling 100 meters from the strip's start (Photo: Dr. Gerhard du Preez). B) Five samples per location including the centre point sample (Photo: Dr. Gerhard du Preez)

For each sub-sample, the organic layer was removed and the top 10 cm of soil collected using a spade. After collecting all five sub-samples, the soil was homogenised in a bucket using a hand spade (Sheppard and Addison, 2008). Two one-litre (L) composite samples were stored in separate zip-lock bags, each labelled with the sampling site name (e.g., NV – Nicol de Vos) and strip number (e.g., 1–27). Filled zip-lock bags were placed in polystyrene cooler boxes to ensure temperature moderation and sunlight blockage. Once all the sampling had been completed, the samples were transported to the North-West University (NWU) and stored at 6 °C (Sheppard & Addison, 2008) until further processing.

3.4. SOIL SAMPLE PREPARATION

The reason why two composite samples were collected, was to facilitate the analysis of physical, chemical, and biological soil health parameters at two separate locations. Soil texture (a physical parameter) and permanganate-oxidisable carbon (POXC) (a biological parameter) were analysed at the NWU. All other parameters were analysed during the Haney Analysis (Annexure D) at Ward Laboratories Inc. in the United States (USA).

For preparation of analysis at the NWU, the samples were first transported from the labelled zip-lock bags to containers designated for air drying. Samples were air-dried for a minimum of 72 hours in bowls labelled according to each ecotope. Once the soil had reached dry mass, the samples were lightly crushed and sieved through a 2mm mesh as required by protocol (Schindelbeck et al. 2016). The sieved samples were then placed in 100 ml plastic air-tight containers, appropriately marked, and stored in polystyrene cooler boxes until testing.

For the Haney analysis, samples were stored in the same zip-lock bags used during sampling and placed in separate isolation coolers for each ecotope before being sent to the Soil Health Support Centre (<https://www.soilhealthlab.co.za/index.php/tests-offered/>). From there, the samples were forwarded to Ward Laboratories Inc. for further analysis. Each soil sample was dried at 50°C, sieved to pass through a 2 mm mesh, and divided into multiple subsamples in preparation for further analysis.

3.5. ANALYSIS OF PHYSICAL PARAMETERS

In this study, the rapid soil texture analysis was performed at the NWU to determine soil particle size distribution, following the method outlined by Schindelbeck et al. (2016). Additionally, volumetric aggregate stability measurements were conducted to assess the stability of

macroaggregates and microaggregates, following Standard Operating Procedure No. 9 from Regenerative Agriculture Labs LLC (subcontracted by Ward Laboratories Inc.).

3.5.1. Soil texture

During the rapid soil texture analysis (Schindelbeck et al. 2016), the soil particles were dispersed in a 3% sodium hexametaphosphate solution, and the suspension was transferred to a 0.053mm sieve to separate the sand fraction, which was then collected, dried, and weighed. The remaining suspension was allowed to settle for two hours to separate the silt from the clay. The silt fraction was carefully decanted, dried, and weighed. The clay content was determined by subtracting the combined weights of sand and silt from the total soil weight (Schindelbeck et al. 2016).

3.5.2. Volumetric aggregate stability

The measurement of volumetric aggregate stability in soil was conducted to assess the stability of macroaggregates (greater than 0.25 mm) and microaggregates (between 0.25 mm and 0.053 mm), providing insights into soil structure and resilience. The prepared samples underwent a wet sieving process using a 0.25 mm sieve, where they were submerged in deionised water and agitated to isolate the macroaggregates. The retained material was treated with sodium hexametaphosphate to break down the aggregates further, allowing for the separation of finer particles. This process was repeated with a 0.053 mm sieve to collect the microaggregate fraction. Both fractions were subsequently dried, and their volumes were measured. The volumetric aggregate stability was calculated by combining the volumes of stable macroaggregates and microaggregates (Schindelbeck et al. 2016).

3.6. ANALYSIS OF CHEMICAL PARAMETERS

Chemical properties were analysed according to Standard Operating Procedure No. H1 used by Ward Laboratories Inc. and Regen Ag Lab LLC (Haney et al. 2018). Measurements were conducted using both water and H3A extracts to provide insights into nutrient availability and soil pH dynamics. The H3A solution mimics organic acids produced by plant roots, temporarily lowering soil pH to enhance nutrient solubility. It was prepared by dissolving 11.0 g of malic acid, 7.0 g of citric acid, and 4.5 g of oxalic acid in 10 L of deionised water, adjusted to a pH of approximately 3.75.

3.6.1. Soil pH

Soil pH was measured using the 1:1 water pH method (Miller et al. 2013), where 10 grams of soil were mixed with 10 mL of water and allowed to react for 30 minutes. Electrical conductivity (EC) was also measured to assess soluble salts, while the soil pH was used to classify the soil as alkaline, neutral, or acidic. For samples with an acidic pH (<6.5), a buffer solution was added to determine total acidity, enabling the laboratory to estimate the lime required to neutralise the soil's acidity.

3.6.2. Soil nutrients

The H3A extract was used on a 4 g subsample of soil mixed with 40 mL of H3A, shaken for 10 minutes, centrifuged, and filtered before analysis. After extraction, the nutrient content of both H3A and water extracts was measured using the Lachat 8000 flow injection analyser (Hach Company) and a Thermo Scientific Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). The Lachat analyser measured inorganic nitrogen ($\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$) and phosphate ($\text{PO}_4\text{-P}$) concentrations. Meanwhile, the ICP-OES assessed a wide range of macronutrients and micronutrients in the H3A extract, including phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), sulphur (S), and aluminium (Al).

3.6.3. Total Organic Carbon and Total Nitrogen

Total Organic Carbon and Total Nitrogen in the water extract were quantified using the Teledyne-Tekmar Torch C analyser, which offers detailed measurements in parts per million (ppm) for assessing nutrient availability.

3.6.4. Nitrogen and Phosphorus Calculations

The Total Nitrogen (Organic + Inorganic) value was derived by summing inorganic nitrogen ($\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$) and organic nitrogen values from both water and H3A extracts. Additionally, the Organic N: Inorganic N Ratio were calculated to assess the soil's nitrogen balance, important for both immediate and potential microbial nutrient use. Organic nitrogen release and reserve metrics estimated the amount of nitrogen that can mineralise over time. Phosphorus analysis included values for Inorganic Phosphorus, Organic Phosphorus, and Total Phosphorus in ppm. These are

further used to calculate P Saturation ratios with aluminium/iron and calcium, indicating phosphorus availability and buffering capacity.

3.7. ANALYSIS OF BIOLOGICAL PARAMETERS

In measuring POXC, the standard operating procedures of the Cornell University were performed at the NWU as described by Schindelbeck et al. (2016). Further analysis of biological parameters was done by Ward Laboratories Inc. and Regen Ag Lab LLC (Miller et al. 2013).

3.7.1. Permanganate oxidisable carbon (POXC) analysis

Permanganate oxidisable carbon was measured by using a 0.2 M potassium permanganate (KMnO₄) solution prepared and adjusted to pH 7.2. A standard curve was created by measuring absorbance for varying KMnO₄ concentrations using a calibrated spectrophotometer. Soil samples (2.5 g) were mixed with the KMnO₄ solution, shaken, and allowed to react for 10 minutes. After dilution, absorbance was measured, and results were used to calculate POXC by comparing absorbance against the standard curve. POXC concentration was calculated using a linear regression formula (Equation 3.7.1) while the active carbon content in soil (mg/kg) was calculated based on the initial KMnO₄ concentration, soil weight, and the amount of carbon oxidised (Equation 3.7.2) Samples were retested if replicate absorbance values differed by more than 5%.

Equation 3.7.1: The formula used to determine concentration

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

Equation 3.7.2: The formula used to calculate the amount of POXC

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

3.7.2. Soil organic matter

Soil Organic Matter (SOM) was measured using the loss on ignition (LOI) method (Miller et al. 2013). A 2 g soil sample was dried and weighed (recorded as WD), then placed in an asher oven for two hours and fifteen minutes at high temperatures to combust the organic material. After

cooling, samples were reweighed (recorded as W_A). The percentage of SOM was calculated (Equation 3.7.3).

Equation 3.7.3: Soil Organic Matter represented as loss on ignition (LOI) percentage

$$\text{LOI (\%)} = (W_D - W_A / W_D) \times 100$$

* W_D – Dry Weight; W_A – Ash Weight

3.7.3. Soil respiration ($\text{CO}_2 - \text{C}$)

To measure Soil Respiration ($\text{CO}_2\text{-C}$), which reflects microbial activity, 40 g soil subsamples were placed in perforated 50 mL plastic beakers and brought to field capacity through capillary action. This setup aimed to simulate natural drying and wetting conditions, which are critical for accurate microbial activity measurement. The beakers were incubated for 24 hours at 24°C , and CO_2 concentrations were then analysed using the SR-1 soil respiration system from Soil Health Innovations. The SR-1 system, equipped with either the LI-COR 840A or LI-COR 850 infrared gas analyser (IRGA), measured $\text{CO}_2\text{-C}$, and custom software reported results in parts per million (ppm). High CO_2 emissions correlated with increased microbial activity, which is essential for nutrient cycling.

3.7.4. Water Extractable Organic Carbon (WEOC) and Water Extractable Organic Nitrogen (WEON)

Water Extractable Organic Carbon and WEON were analysed to estimate the labile carbon and nitrogen pools readily available for microbial consumption. A 4 g soil sample was mixed with 40 mL of deionised water, shaken for 10 minutes, centrifuged at 3000 rpm for 5 minutes, and filtered through Whatman 2V filter paper. The filtered solution was analysed using a Total Organic Carbon (TOC) analyser, specifically the Teledyne-Tekmar Torch C analyser, which measured WEOC and WEON in parts per million (ppm).

3.8. DETERMINATION OF THE SOIL HEALTH SCORE

After analysis of soil respiration, WEOC and WEON, the numerical soil health score was calculated based on nutrient cycling by using Equation 3.8.1.

$$\text{SHS} = (24 \text{ h CO}_2 \text{ ©} / 10) * (\text{WEOC} / 100) * (\text{WEON} / 10)$$

This calculation considers the general 10:1 carbon: nitrogen ratio of soil, as well as the role of microbial activity in soil nutrient cycling (Haney et al. 2018). By combining soil biological and chemical properties a score was derived that can be used as a monitoring medium over time.

3.9. STATISTICAL ANALYSIS OF DATA

This study employed Permutational Multivariate Analysis of Variance (PERMANOVA) and Principal Component Analysis (PCA) to examine the impact of cropping seasons, ecotopes, and farming systems on soil health. Furthermore, pairwise comparisons were used the test for significant differences ($p < 0.05$) in soil health parameters across seasons, ecotopes, and farming systems. Distance-based linear models were employed to identify best subset (proxy) indicators of soil health.

The PERMANOVA analysis was conducted using Primer 6 (PRIMER-E) software. This technique integrates selected dissimilarity measures within an ANOVA framework, with p-values derived from permutation methods, as described by Anderson (2017). Combining the partitioning capabilities of traditional ANOVA with the rank-based properties of nonparametric methods such as Analysis of Similarities (ANOSIM), it allows for flexible, distribution-free inferences. The analysis included three fixed factors: “cropping season” (three levels: 1-2021, 2-2022, and 3-2023), “ecotope” (three levels: Hendrik Odendaal, Nicol de Vos, and Nant Yzel), and “farming system” (four levels: conventional, conservation, regenerative agriculture, and natural veld). This comprehensive approach enabled the identification of each factor’s effects on soil health, as well as their interactions.

Additionally, ordination analyses were conducted using Principal Component Analysis (PCA) with all soil health parameters, utilising Primer 6 (PRIMER-E) software. This analysis is a statistical method that reduces dataset dimensionality while preserving maximum variability, transforming the original correlated variables into a set of uncorrelated principal components (PCs), with the first few capturing the most variation. This process identifies eigenvectors of the covariance matrix, indicating directions of maximum variance (Jolliffe, 2002). Each soil health parameter was correlated with the main PC axes (PC1 and PC2), which accounted for most of the variation, and those parameters with the strongest correlation ($r > 0.7$ or $r < -0.7$) were represented as vectors on graphs, clearly highlighting the main soil health parameters. Although multiple parameters

showed correlations in each cropping season, this study focused on those with the strongest correlations to the PC axes. Once these main parameters were identified, any remaining parameters with weaker correlations were tested for co-correlation and, if present, were excluded from pairwise comparisons to avoid redundancy.

After identifying the main soil health parameters, pairwise comparisons were conducted for each parameter following the same design as the multivariate approach. This method enabled the identification of variations in soil health parameters between ecotopes and farming systems within each cropping season. Ecotopes were compared only within each season, as were farming systems within each ecotope, to focus on identifying significant differences ($p < 0.05$) and trends specific to individual seasons. Testing across cropping seasons was not pursued, as similar trends were consistently observed in the PCAs, justifying a season-by-season approach for clarity and precision in results.

The soil health score from the Haney Analysis was assessed separately from the main soil health parameters in pairwise comparisons. This separate analysis was conducted because the soil health score provides an overall indicator of soil health, rather than focusing on individual parameters. By comparing the soil health score independently, it was possible to determine whether the implementation of different farming systems had a direct impact on overall soil health, beyond just the parameters that contribute to it.

Additionally, all the measured physical, chemical, and biological soil health parameters were tested as predictor variables in a distance-based linear model (DB-LM) against Soil Health Scores from each ecotope during the three cropping seasons. This approach was chosen because DB-LM can project data into a Euclidean space based on the distance matrix of predictors, which accommodates mixed-type variables (Boj & Grané, 2024). This analysis identified specific soil health parameters most closely associated with the derived soil health scores, allowing these parameters to serve as proxy indicators. To avoid redundancy, parameters already used in calculating the soil health score, namely, water-extractable organic carbon, nitrogen, and carbon dioxide respiration, were excluded.

CHAPTER 4 RESULTS

4.1. INFLUENCE OF CROPPING SEASON, ECOTOPES AND FARMING SYSTEMS ON SOIL HEALTH PARAMETERS

Permutational Multivariate Analysis of Variance (PERMANOVA) was performed to evaluate whether cropping season, ecotope, and farming system influenced the measured soil health parameters (physical, chemical, and biological). The analysis showed that each factor, as well as their two-way and three-way interactions, had a significant effect on soil health parameters ($p < 0.0001$). However, this result only indicates that these factors and their interactions influenced soil health, without specifying which parameters were significantly influenced. To identify the main soil health parameters, ordination analyses were conducted using Principal Component Analysis (PCA). Each soil health parameter was correlated with the principal components (PC1 and PC2), with those showing the strongest correlations ($r > 0.7$ or $r < -0.7$) displayed as vectors on the graphs. This method allowed for the identification of the main soil health parameters that contributed most to the variation observed in the study (Annexure E).

Principal components (PC 1 and PC 2) of the first cropping season represents 50% and 18% of the variation in soil health parameters, respectively. Different colours represented each ecotope, showing distinct clustering patterns (Figure 4.1.1). Notably, the HO ecotope was distinctly separated from NV and NY. Within each main cluster, the Uncult system was clearly set apart from the other systems. The main physical parameters responsible for the observed variation in soil health properties were sand ($r = 0.90$ with PC1), clay ($r = -0.95$ with PC1), and volumetric aggregate stability (VAS) ($r = -0.95$ with PC1). Among the chemical parameters, copper (Cu) ($r = 0.90$ with PC1), magnesium (Mg) ($r = -0.88$ with PC1), iron (Fe) ($r = 0.78$ with PC2), and pH ($r = -0.74$) emerged as the main soil health parameters. Additional parameters also showed correlations with PC1 or PC2 but were excluded from the PCA and pairwise comparisons due to co-correlation ($r > 0.7$ or $r < -0.7$) with at least one of the main variables (Annexure F). These included manganese (Mn) ($r = 0.77$ with PC1), zinc (Zn) ($r = 0.73$ with PC1), organic nitrogen (organic N) ($r = -0.85$ with PC1), sulphur (S) ($r = -0.85$ with PC1), sodium (Na) ($r = -0.72$ with PC1), and potassium (K) ($r = -0.73$ with PC2). Organic matter (OM) was recorded as the main biological parameter ($r = -0.95$ with PC1). Soil respiration (CO_2) ($r = -0.95$ with PC1) and total organic carbon (Total Organic C) ($r = -0.93$ with PC1) were identified as additional parameters. They were excluded from the PCA and pairwise comparisons since they co-correlated with OM (Annexure F).

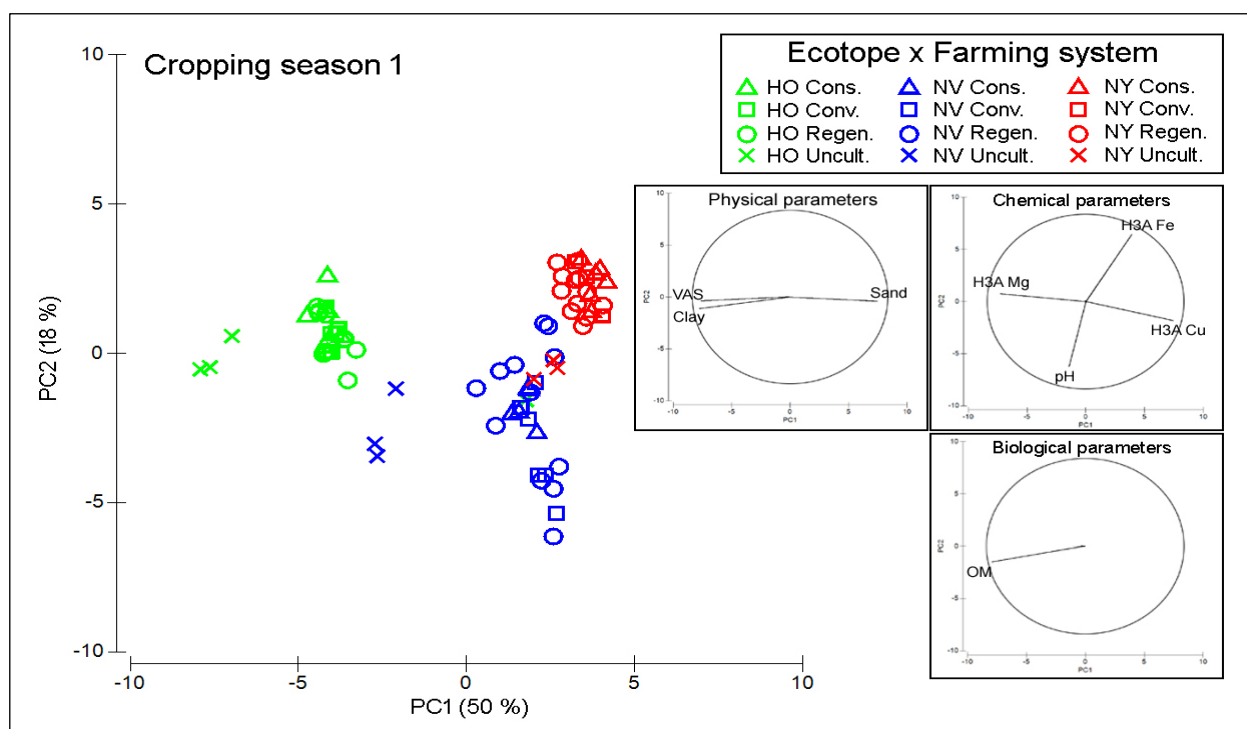


Figure 4.1.1: Principal Component Analysis (PCA) plot illustrating variation in measured physical, chemical, and biological soil health parameters during the first cropping season (2020/2021). Distinct colours denote the three ecotopes: Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY). Different shapes represent the conventional (Conv), conservation (Cons), and regenerative agriculture (Regen) systems, as well as the uncultivated veld (Uncult) system. Overlay plots illustrate the main physical, chemical, and biological parameters with the greatest correlations ($r > 0.7$ or below $r < -0.7$) with the recorded variation in soil health parameters.

The clustering pattern observed during the second cropping season was similar to that of the first (Figure 4.1.2). Principal components (PC1 and PC2) explained 53% and 14% of the variation in soil health parameters, respectively. The HO ecotope remained distinctly separated from NV and NY, and within each main cluster, the Uncult system was clearly set apart from the other systems. The main physical soil health parameters remained consistent in the second cropping season (2021/2022), including sand ($r = 0.80$ with PC1), clay ($r = -0.75$ with PC1), and VAS ($r = -0.87$ with PC1). However, different main chemical parameters were identified, such as Mn ($r = 0.90$ with PC1), S ($r = -0.92$ with PC1), and pH ($r = -0.80$ with PC2). Other chemical parameters like Cu ($r = 0.78$ with PC1), Fe ($r = 0.77$ with PC1), Na ($r = -0.87$ with PC1), Mg ($r = -0.87$ with PC1), and Organic N ($r = -0.83$ with PC1) also showed correlations with PC1 or PC2 but were excluded from the PCA and pairwise comparisons due to co-correlation with the main variables (Annexure G). Organic matter (OM) ($r = -0.93$ with PC1) was the main biological parameter. Additional parameters such as total organic carbon (Total Organic C) ($r = -0.90$ with PC1), soil respiration ($r = -0.72$ with PC1), and permanganate-oxidisable carbon (POXC) ($r = -0.71$ with PC1) were also correlated with PC1 or PC2 but were excluded due to co-correlation (Annexure G).

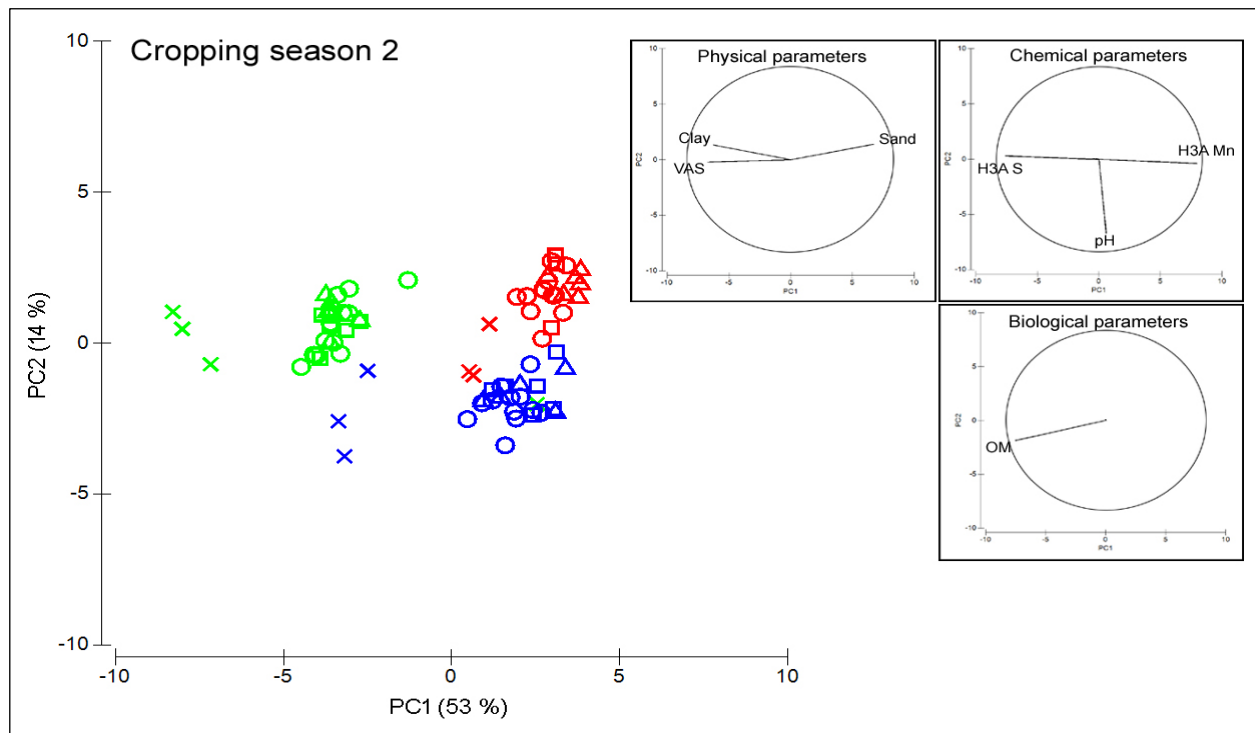


Figure 4.1.2: Principal Component Analysis (PCA) plot illustrating variation in measured physical, chemical, and biological soil health parameters during the second cropping season (2021/2022). Distinct colours denote the three ecotopes: Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY). Different shapes represent the conventional (Conv), conservation (Cons), and regenerative agriculture (Regen) systems, as well as the uncultivated veld (Uncult) system. Overlay plots illustrate the main physical, chemical, and biological parameters with the greatest correlations ($r > 0.7$ or below $r < -0.7$) with the recorded variation in soil health parameters.

Clustering was also observed during the final cropping season (Figure 4.1.3). Principal components (PC 1 and PC 2) of the third cropping season explained 54% and 16% of the variation in soil health parameters, respectively. The HO ecotope remained distinctly separated from NV and NY, and within each main cluster, the Uncult system was still clearly set apart from the other systems. Sand ($r = 0.88$ with PC1), clay ($r = 0.88$ with PC1), and VAS ($r = -0.92$ with PC1) consistently emerged as the main physical soil health parameters. Iron ($r = 0.89$ with PC1), Na ($r = 0.89$ with PC1), and inorganic nitrogen (Inorg. N) ($r = -0.93$ with PC2) were identified as the main chemical soil health parameters. Manganese ($r = 0.88$ with PC1), Cu ($r = 0.76$ with PC1), S ($r = -0.88$ with PC1), Mg ($R^2 = -0.87$ with PC1), Organic N ($r = -0.87$ with PC1), Total N ($r = -0.72$ with PC1), and total available N ($r = -0.84$ with PC2) were identified as additional parameters, showing correlation with PC1 or PC2 but were excluded from the PCA and pairwise comparisons due to co-correlation with at least one of the main variables (Annexure H). Just as the previous cropping season, OM ($r = -0.93$ with PC1) was identified as the main biological soil health parameter. However, total organic C ($r = -0.90$ with PC1) was also identified as an additional parameter which also presented correlations to PC 1 or PC 2 but were excluded from the PCA and pairwise comparisons due to co-correlation (Annexure H).

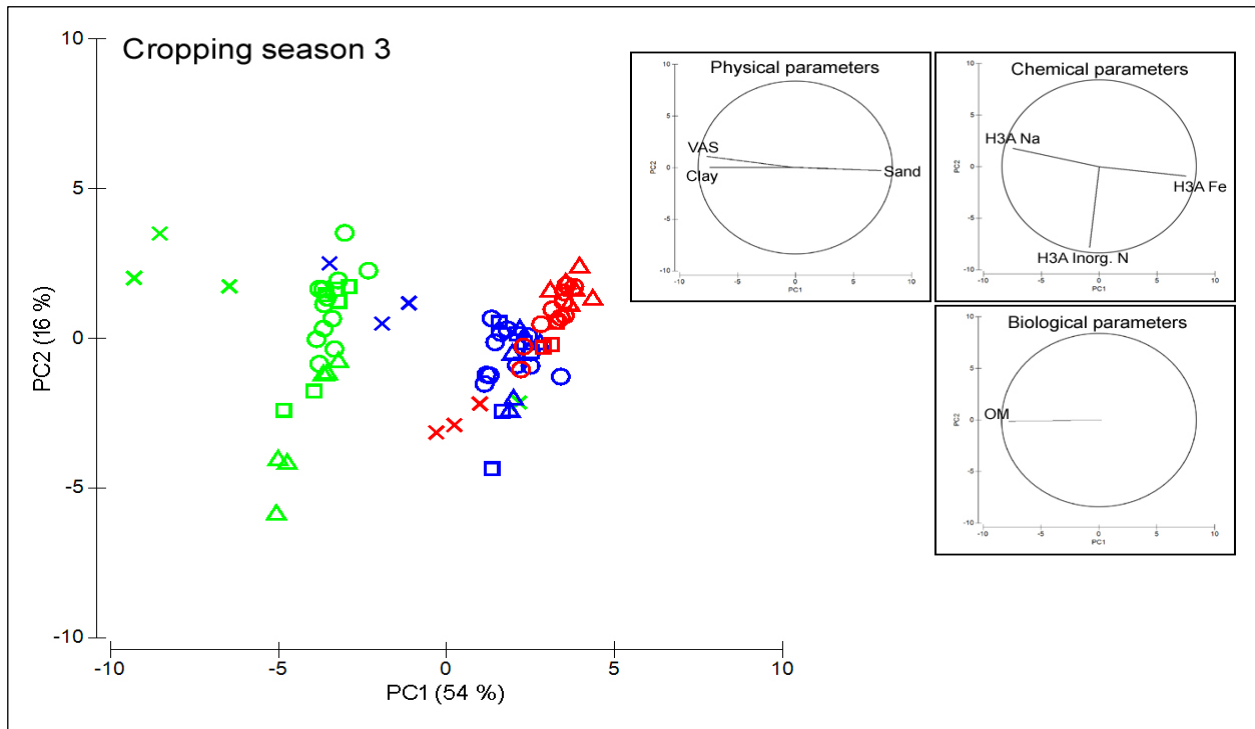


Figure 4.1.3: Principal Component Analysis (PCA) plot illustrating variation in measured physical, chemical, and biological soil health parameters during the third sampling cropping season (2022/2023). Distinct colours denote the three ecotopes: Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY). Different shapes represent the conventional (Conv), conservation (Cons), and regenerative agriculture (Regen) systems, as well as the uncultivated veld (Uncult) system. Overlay plots illustrate the main physical, chemical, and biological parameters with the greatest correlations ($r > 0.7$ or below $r < -0.7$) with the recorded variation in soil health parameters.

4.2. COMPARISON OF MAIN SOIL HEALTH PARAMMETERS

In this study, principal component analysis (PCA) identified the main soil health parameters based on their contribution to variation across ecotopes and farming systems. These parameters showed the strongest correlations with the principal components and were considered the primary drivers of the observed changes. Additional parameters, although correlated with PC1 or PC2, were co-correlated with the main parameters, meaning their variations were closely linked. Including them in pairwise comparisons would have been redundant, as their patterns mirrored the main parameters.

4.2.1. Physical parameters

Sand content was clearly differentiated between ecotopes in each cropping season (Figure 4.2.1). In the first cropping season, sand content at HO ($21 \pm 4\%$) significantly differed from NV ($70 \pm 14\%$) and NY ($79 \pm 3\%$), with NV and NY also significantly differing from each other. No significant differences were observed between farming systems at HO during this season. However, at NV,

the Uncult system ($52 \pm 3\%$) significantly differed from the implemented farming systems (Conv – $75 \pm 3\%$, Cons – $75 \pm 4\%$, Regen – $69 \pm 19\%$). A similar pattern was seen at NY, where the Uncult system ($73 \pm 2\%$) significantly differed from Conv ($80 \pm 2\%$), Cons ($80 \pm 2\%$), and Regen ($79 \pm 2\%$). The significant differences between HO, NV, and NY persisted through the second cropping season, with the only exceptions between NV and NY occurring in the Regen and Uncult systems, which did not significantly differ. Similarly, the Uncult system continued to significantly differ from the implemented systems at both HO and NV. In the final cropping season, HO, NV, and NY remained significantly different, with exceptions noted between NV and NY for the Uncult systems. The only significant differences between farming systems were observed in the Uncult systems, which differed from the implemented systems at HO and NY.

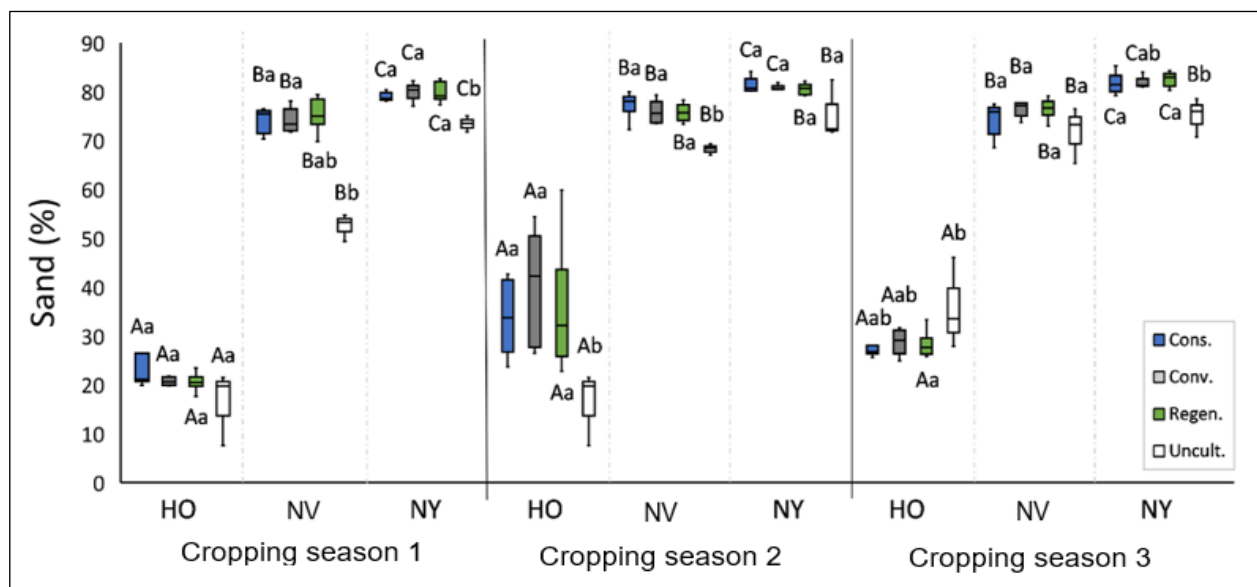


Figure 4.2.1: Pairwise comparisons ($p < 0.05$) of sand content (%) for all three cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$) differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively.

Clay content also presented a clear distinction between ecotopes during all three cropping seasons (Figure 4.2.2). Specifically, HO ($42 \pm 2\%$) significantly differed from NV ($15 \pm 5\%$) and NY ($7 \pm 1\%$) during the first cropping season. Consistently the Uncult systems from HO ($47 \pm 4\%$), NV ($27 \pm 4\%$), and NY ($8 \pm 1\%$) significantly differed from the implemented farming systems at HO (Conv – $41 \pm 3\%$, Cons – $41 \pm 1\%$, Regen – $41 \pm 1\%$), NV (Conv – $13 \pm 2\%$, Cons – $13 \pm 2\%$, Regen – $13 \pm 2\%$), and NY (Conv – $7 \pm 1\%$, Cons – $7 \pm 1\%$, Regen – $7 \pm 1\%$) respectively. The significant differences between HO, NV, and NY persisted through the second cropping season, with no significant differences observed between the farming systems at HO. However, at NV,

both the Uncult and the Regen system ($14 \pm 2\%$) significantly differed from the Conv system ($14 \pm 2\%$). At NY, only the Uncult system significantly differed from the implemented farming systems. In the final cropping season, HO, NV, and NY remained significantly different from one another, with the only notable difference between systems being the Uncult system at NY, which significantly differed from the implemented systems.

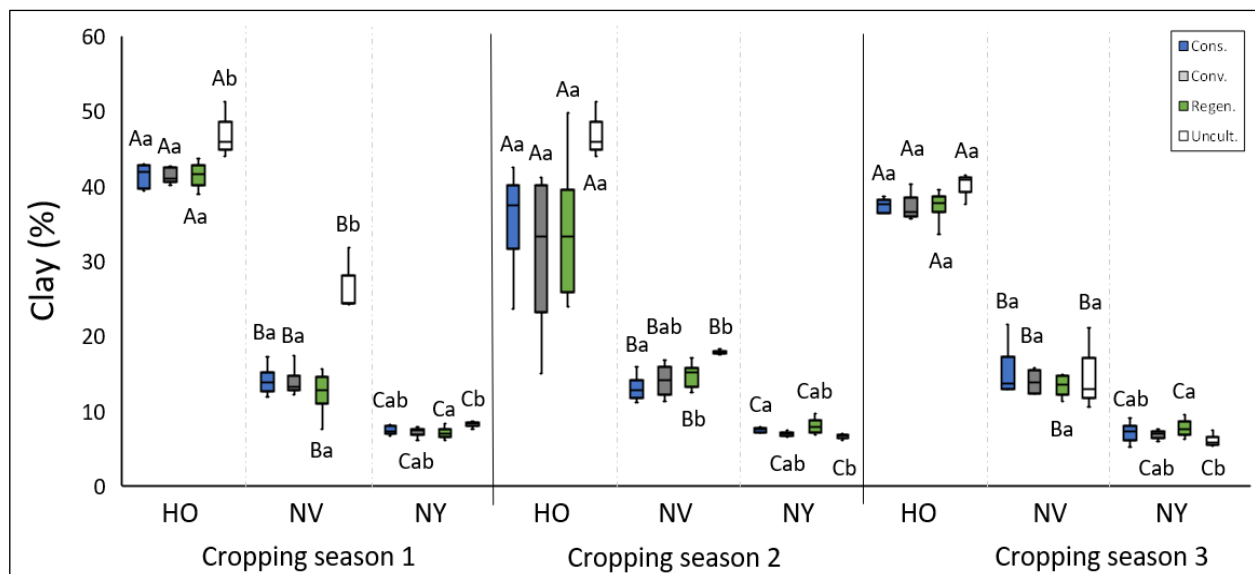


Figure 4.2.2: Pairwise comparisons ($p < 0.05$) of clay content (%) for all three cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$) differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively

Volumetric aggregate stability had the same trend in distribution as clay content (Figure 4.2.3). Hendrik Odendaal ($24 \pm 6\%$) significantly differed from NV ($5 \pm 3\%$) and NY ($2 \pm 1\%$) during the first cropping season. Additionally, NV and NY significantly differed from one another, except for the Cons systems. There were no significant differences between the farming systems at HO, but at NV, the Uncult system ($10 \pm 2\%$) significantly differed from the implemented farming systems (Conv – $4 \pm 1\%$, Cons – $3 \pm 2\%$, Regen – $4 \pm 2\%$). While at NY, the Uncult system (mean 2.8%) significantly differed from both the Cons ($3 \pm 1\%$) and Regen systems ($2 \pm 0.2\%$), which also significantly differed from each other. During the second cropping season, HO ($52 \pm 14\%$) remained significantly different from NV ($11 \pm 17\%$), except for the Uncult systems, which did not show significant differences. Hendrik Odendaal was also significantly different from NY ($7 \pm 13\%$) across all systems. It was however noted that NV and NY did not significantly differ. There were no significant differences between systems at HO. Only the Uncult system ($54 \pm 3\%$) at NV were

noted to significantly differ from the implemented systems (Conv – $5 \pm 2\%$, Cons – $3 \pm 3\%$, Regen – $8 \pm 10\%$). The same trend was noticed at NY. During the final cropping season, HO ($37 \pm 13\%$), NV ($8 \pm 14\%$), and NY ($4 \pm 5\%$) were all significantly different from one another, except for the Uncult systems, which did not show significant differences between any of the ecotopes. At HO, the only significant difference between farming systems was between the Uncult system ($53 \pm 9\%$) and the Conv system ($32 \pm 12\%$). At NV, only the Uncult system significantly differed from the implemented farming systems, while at NY, the Uncult system significantly differed from both the Conv and Regen systems.

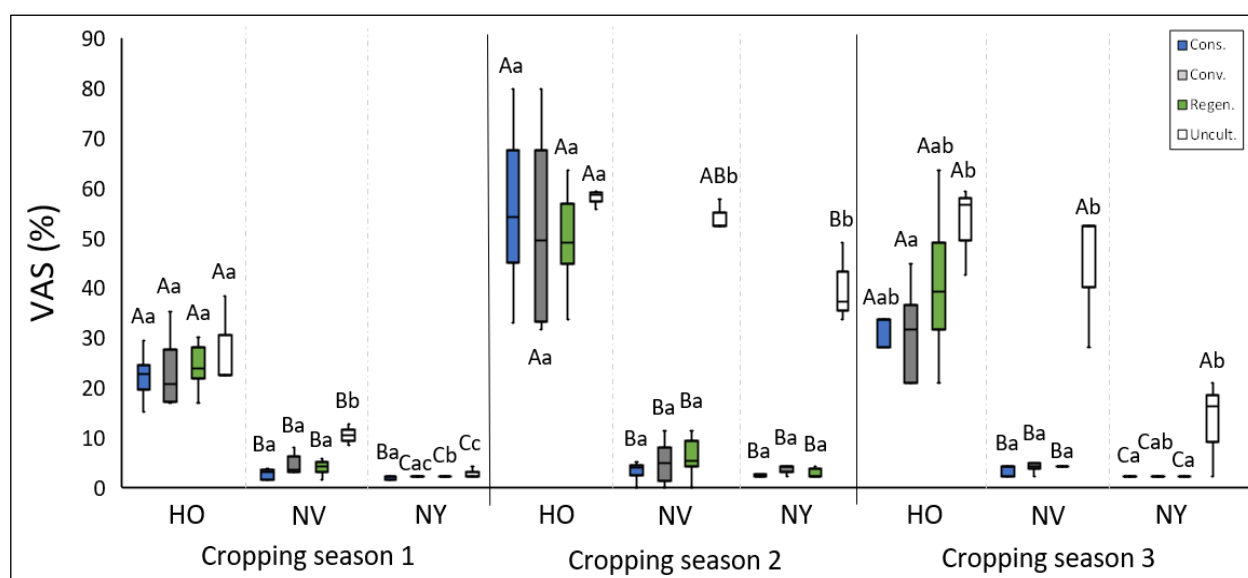


Figure 4.2.3: Pairwise comparisons ($p < 0.05$) of volumetric aggregate stability (%) for all three cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$) differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively

4.2.2. Chemical parameters

A similar trend was observed by Cu during each of the cropping seasons (Figure 4.2.4). During the first cropping season, HO (0.33 ± 0.07 ppm) significantly differed from NV (0.85 ± 0.24 ppm) (except for the Uncult systems) and NY (0.90 ± 0.10 ppm). Between NV and NY, there were only two significant differences: the Conv (0.90 ± 0.10 ppm) and Regen (0.80 ± 0.10 ppm) systems. At HO, the only significant difference between systems was the Uncult system (0.20 ± 0.09 ppm) compared to the implemented farming systems (Conv – 0.33 ± 0.04 ppm, Cons – 0.35 ± 0.05 ppm, Regen – 0.36 ± 0.07 ppm). Similarly, at NV, only the Uncult system (0.45 ± 0.29 ppm) significantly differed from the implemented systems (Conv – 1.02 ± 0.12 ppm, Cons – 1.06 ± 0.10 ppm, Regen – 0.98 ± 0.19 ppm). At NY, the Conv and Regen systems significantly differed from

the Cons and Regen systems. During the second cropping season, HO significantly differed from both NV and NY. The trend between NV and NY remained similar, but the NV Cons system (1.10 ± 0.10 ppm) now significantly differed from the NY Cons system (0.80 ± 0.00 ppm). In the final cropping season, all three ecotopes showed significant differences from one another. Between farming systems HO, the Uncult system remained significantly different from the Cons and Regen farming systems. At NV, only the Uncult system significantly differed from the Regen system. At NY, the Uncult system significantly differed from both the Cons and Conv systems, with the Cons and Conv systems also significantly differing from each other.

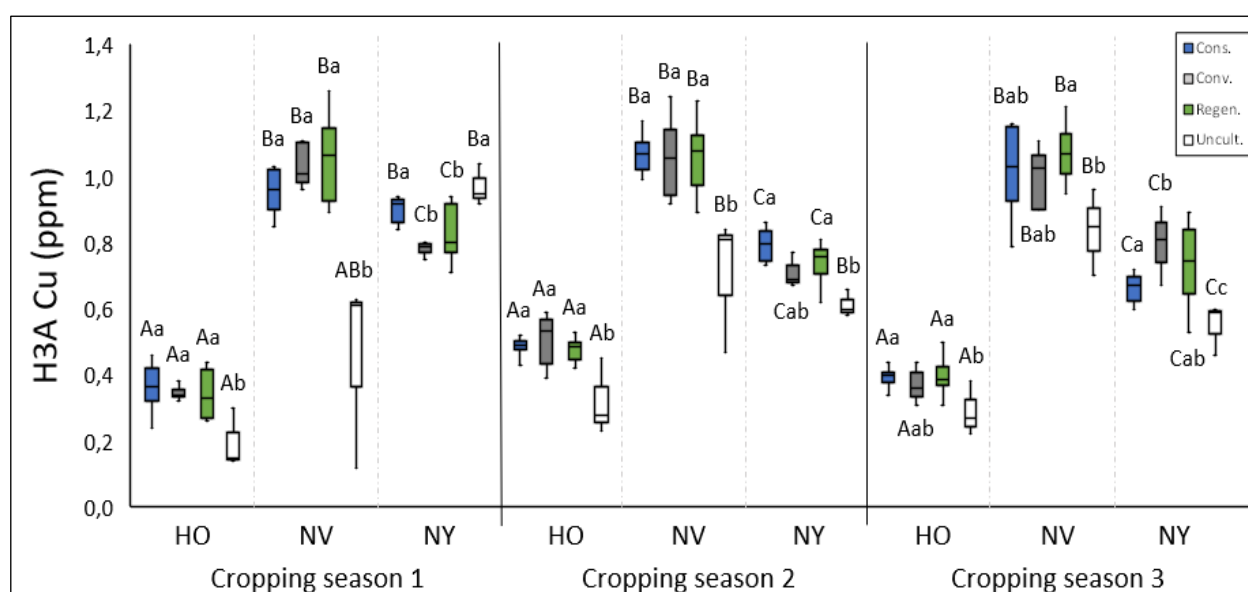


Figure 4.2.4: Pairwise comparisons ($p < 0.05$) of H3A copper (Cu) (parts per million; ppm) for all three cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$) differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively

Magnesium showed slight fluctuations across the three cropping seasons (Figure 4.2.5). In the first season, HO (181 ± 22 ppm) significantly differed from NV (68 ± 32 ppm) (except for the Uncult systems) and from NY (44 ± 22 ppm). The Cons (63 ± 25 ppm) and Regen (68 ± 32 ppm) systems at NV also significantly differed from the Cons (37 ± 4 ppm) and Regen (38 ± 5 ppm) systems at NY. At HO, significant differences between farming systems were observed, with the Uncult system (231 ± 11 ppm) differing from both the Conv (178 ± 8 ppm) and Cons (177 ± 9 ppm) systems, which also differed from each other. There were no significant differences between farming systems at NV, while at NY, only the Uncult system (93 ± 45 ppm) significantly differed from the implemented farming systems. During the second cropping season, HO significantly differed from NV (except for the Uncult systems) and from NY, while NV and NY completely

significantly differed from each other. At HO and NV, the only significant difference between farming systems were between the Uncult system and the implemented farming systems. At NY, the Uncult system significantly differed from the Cons and Regen systems. In the final cropping season, the significant differences between ecotopes remained unchanged. The trend in significant differences between farming systems was consistent for HO and NV, while at NY, the Uncult system significantly differed from the implemented systems, and the Conv system (26 ± 1 ppm) also significantly differed from the Cons (28 ± 2 ppm) and Regen (31 ± 3 ppm) systems.

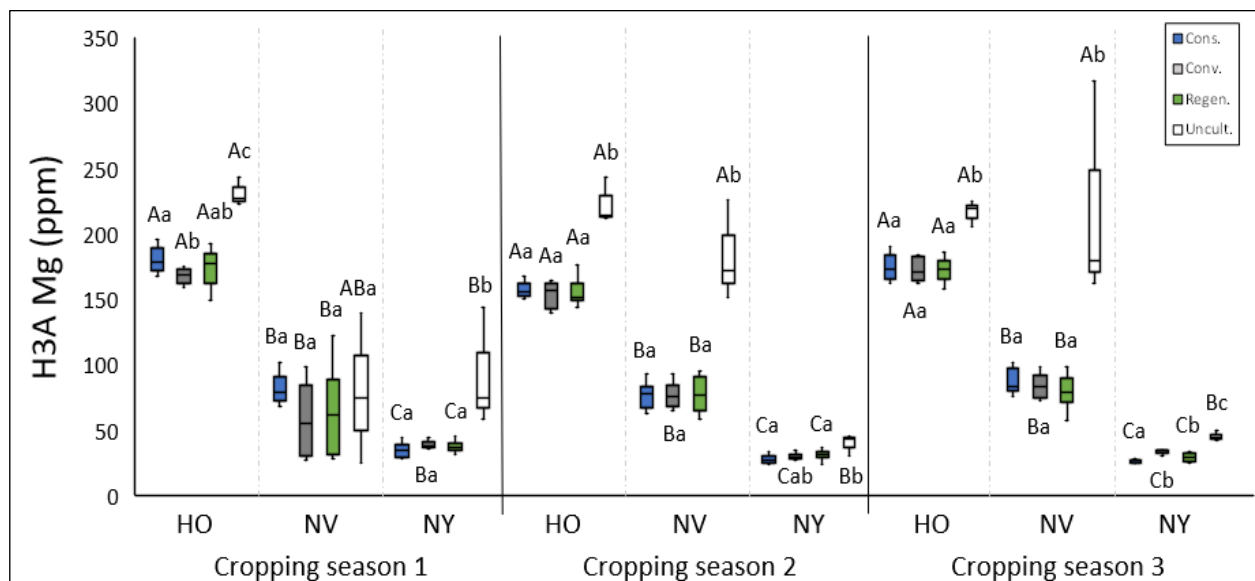


Figure 4.2.5: Pairwise comparisons ($p < 0.05$) of H3A magnesium (Mg) (parts per million; ppm) for all three cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$) differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively.

Iron fluctuated between ecotopes and farming systems over the three cropping seasons, with notable variability in the Regen and Uncult systems, particularly at NV and NY (Figure 4.2.6). During the first cropping season, the only significant difference between HO and NV was in the Cons system, with values of 80 ± 6 ppm and 93 ± 70 ppm, respectively. However, HO (75 ± 16 ppm) significantly differed from NY (171 ± 31 ppm), except for the Uncult system, and NV significantly differed from NY across all systems. No significant differences were found between farming systems at HO. Only the Uncult system (40 ± 11 ppm) significantly differed from the Cons system at NV. At NY, the Uncult system (106 ± 7 ppm) significantly differed from the implemented systems (Conv – 176 ± 30 ppm, Cons – 184 ± 28 ppm, Regen – 179 ± 11 ppm), with the Cons and Regen systems also significantly differing from one another. During the second cropping season, more significant differences were observed between HO and NV, with only the Uncult

systems not significantly differing. Hendrik Odendaal and NY completely significantly differed from one another. Nicol de Vos and NY significantly differed from one another, where the only similarity was in the Conv systems, with values of 127 ± 10 ppm and 172 ± 9 ppm, respectively. At HO, the only significant difference between farming systems was the Uncult system compared to the implemented systems. At NV, the Uncult system significantly differed from both the Conv and Regen systems, while at NY, only the Uncult and Regen systems showed a significant difference. In the final cropping season, the significant differences between HO and NV, as well as between HO and NY, remained unchanged. The only significant difference between NV and NY was in the Conv systems, with values of 143 ± 8 ppm and 171 ± 33 ppm, respectively. The significant differences between farming systems at HO also remained the same. At NY, only the Uncult and Regen systems significantly differed, while no significant differences were observed between farming systems at NV.

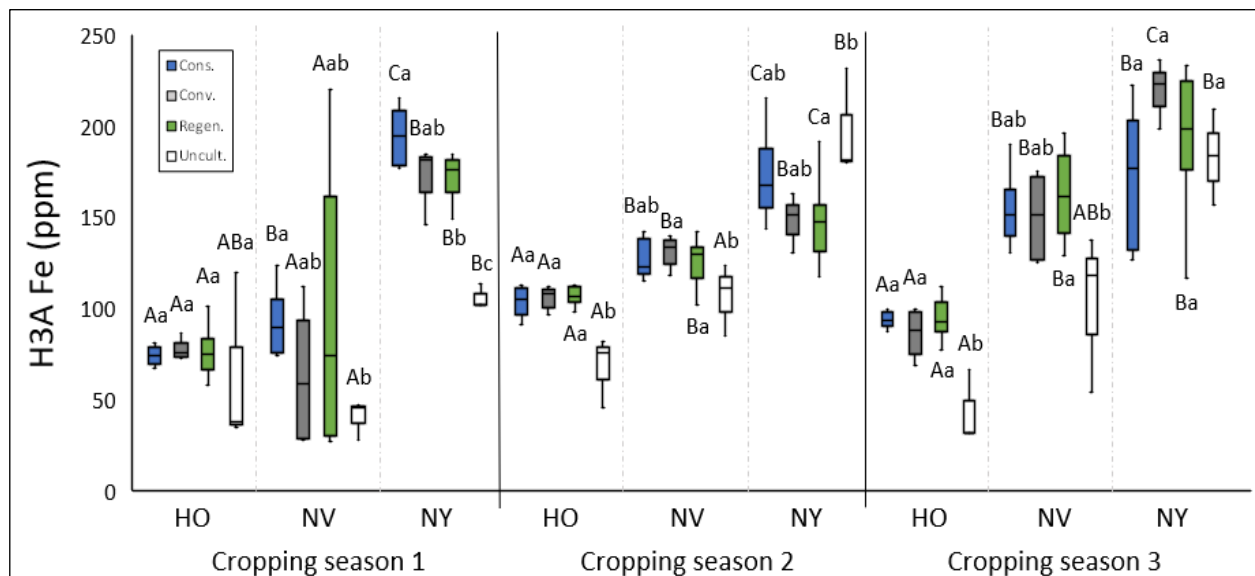


Figure 4.2.6: Pairwise comparisons ($p < 0.05$) of H3A iron (Fe) (parts per million; ppm) for all three cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$) differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively.

Figure 4.2.7 shows that pH values vary between ecotopes and cropping systems over the three cropping seasons. During the first cropping season, HO (5.1 ± 0.4) significantly differed from NV (6.0 ± 0.3) and NY (4.5 ± 0.4), excluding the Uncult systems. Additionally, NV and NY also significantly differed from each other, again excluding the Uncult systems. At HO, the only significant difference between farming systems was the Uncult system (5.9 ± 0.6) compared to the implemented systems (Conv – 5.1 ± 0.2 , Cons – 5.0 ± 0.2 , Regen – 5.0 ± 0.2). No significant

differences were found between farming systems at NV. At NY, the Uncult system (5.5 ± 0.1) significantly differed from the implemented systems (Conv – 4.1 ± 0.2 , Cons – 4.3 ± 0.1 , Regen – 4.4 ± 0.2), with the Regen system also significantly differing from both the Conv and Cons systems. During the second cropping season, HO significantly differed from NV, except for the Uncult system. Between HO and NY, only the Uncult systems showed a significant difference. The significant differences between NV and NY remained unchanged. Between farming systems at HO, the Uncult system continued to significantly differ from the implemented farming systems. At NV, the Uncult system significantly differed from the Cons and Regen systems. The only significant difference at NY was between the Uncult and Cons systems. In the final cropping season, all three ecotopes were significantly different, except for the Uncult systems between HO and NV. The only significant difference between farming systems at HO was between the Uncult system and the implemented farming systems. At NV, no significant differences were found between systems. At NY, the Uncult system significantly differed from the implemented systems, and the Cons and Regen systems also significantly differed from each other.

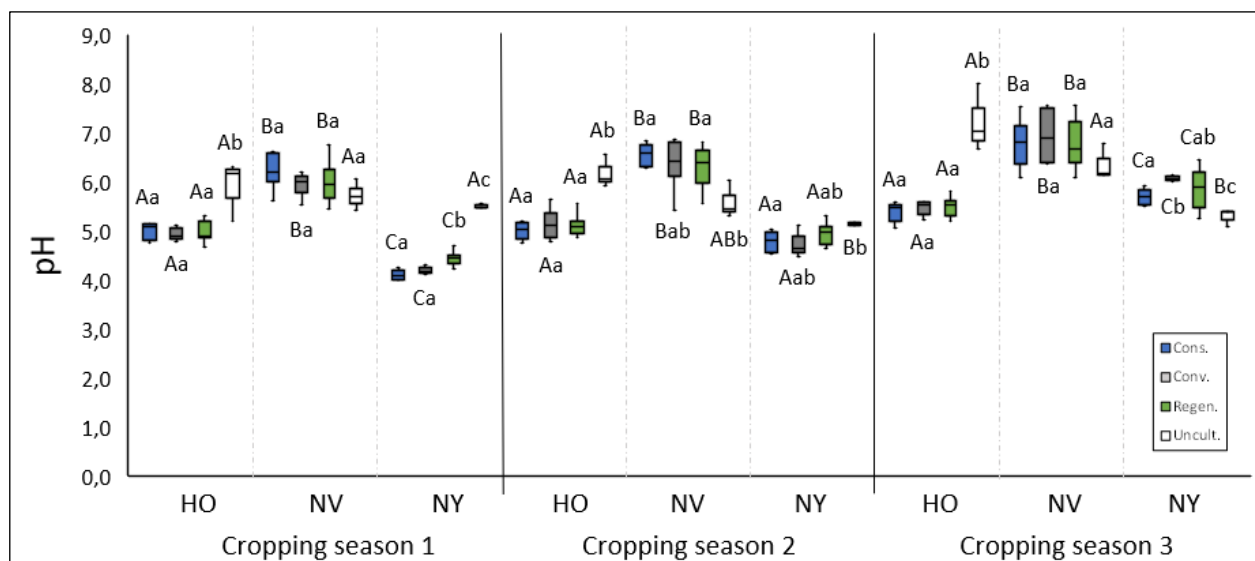


Figure 4.2.7: Pairwise comparisons ($p < 0.05$) of pH for all three cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$) differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively.

Manganese (Figure 4.2.8) showed that only the Cons (5.9 ± 0.9 ppm) and Regen (4.9 ± 1.3 ppm) systems at HO significantly differed from the Cons (33.2 ± 15.6 ppm) and Regen (35.7 ± 18.6 ppm) systems at NV during the first cropping season. Hendrik Odendaal (5.8 ± 2.3 ppm) also significantly differed from NY (26.0 ± 6.2 ppm), except for the Uncult systems. There was no significant difference between NV and NY. At HO, the only significant difference between farming

systems was between the Uncult system (10.6 ± 3.4 ppm) and the implemented systems (Conv – 4.9 ± 0.9 ppm, Cons – 5.9 ± 0.9 ppm, Regen – 4.9 ± 1.3 ppm). Similarly, at NV, the significant difference between the Uncult system and implemented systems were the only significant difference. At NY, the Uncult system significantly differed only from the Cons and Regen systems. During the second cropping season, HO (5.3 ± 1.0 ppm) significantly differed from NV (22.0 ± 7.5 ppm), except the Uncult system. While HO and NY (30.5 ± 8.8 ppm) completely significantly differed from one another. Between NV and NY, only the Regen systems, with values 23.4 ± 5.1 ppm and 35.2 ± 9.2 ppm respectively, as well as the Uncult systems, with values 6.5 ± 2.6 ppm and 19.7 ± 2.9 ppm respectively significantly differed. There were no significant differences between farming systems at HO. Only the Uncult system significantly differed from the implemented systems at NV and NY. During the final cropping season, HO significantly differed from both NV and NY, except the Uncult system. There was no significant difference between NV and NY. The significant differences between farming systems at HO, NV and NY remained the same as observed during the second cropping season.

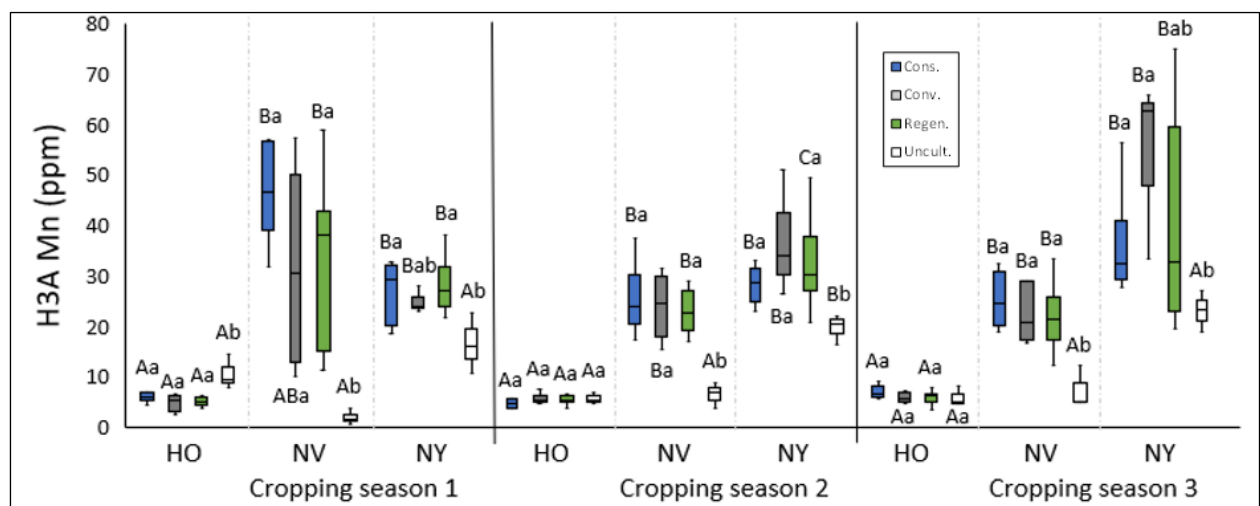


Figure 4.2.8: Pairwise comparisons ($p < 0.05$) of H3A manganese (Mn) (parts per million; ppm) across cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$) differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively.

Sulphur (Figure 4.2.9) indicated that HO (14.4 ± 4.0 ppm) significantly differed from NV (5.7 ± 1.9 ppm) and NY (5.4 ± 1.8 ppm) during the first cropping season. The only significant difference between NV and NY was in the Uncult systems, with values of 8.2 ± 1.4 ppm and 3.0 ± 0.5 ppm, respectively. There were no significant differences between farming systems at HO. At NV, the only significant difference was between the Uncult system and both the Conv (4.8 ± 0.8 ppm) and Cons (4.6 ± 0.3 ppm) systems. At NY, the Uncult system significantly differed from the Cons and

Regen systems. In the second cropping season, HO significantly differed from NV (except for the Uncult system) and was completely different from NY. Between NV and NY, only the Cons and Uncult systems showed significant differences, with values of 4.0 ± 0.6 ppm and 10.0 ± 1.8 ppm for NV, and 2.9 ± 0.8 ppm and 4.4 ± 0.6 ppm for NY. Between farming systems at HO, the Uncult system (27.8 ± 15.0 ppm) significant differed from the implemented farming systems (Conv – 11.4 ± 11.4 ppm, Cons – 10.5 ± 3.4 ppm, Regen – 10.0 ± 1.4 ppm). As did the Cons and Regen systems from one another. At NV, it was only the Uncult system that significantly differed from the implemented farming systems. While at NY, the Cons and Regen (4.1 ± 0.9 ppm) system significantly differed from the Conv system (2.9 ± 0.9 ppm). During the final cropping season, HO significantly differed from NV and NY. It was only the Conv systems between NV and NY that did not significantly differ. Between farming systems at HO, the Uncult system significantly differed from the implemented farming systems. The Cons system also significantly differed from the Conv and Regen systems. At NV, only the Uncult system significantly differed from the Conv and Regen systems. At NY, the Uncult system significantly differed from the implemented farming systems, as did the Cons and Conv systems from one another.

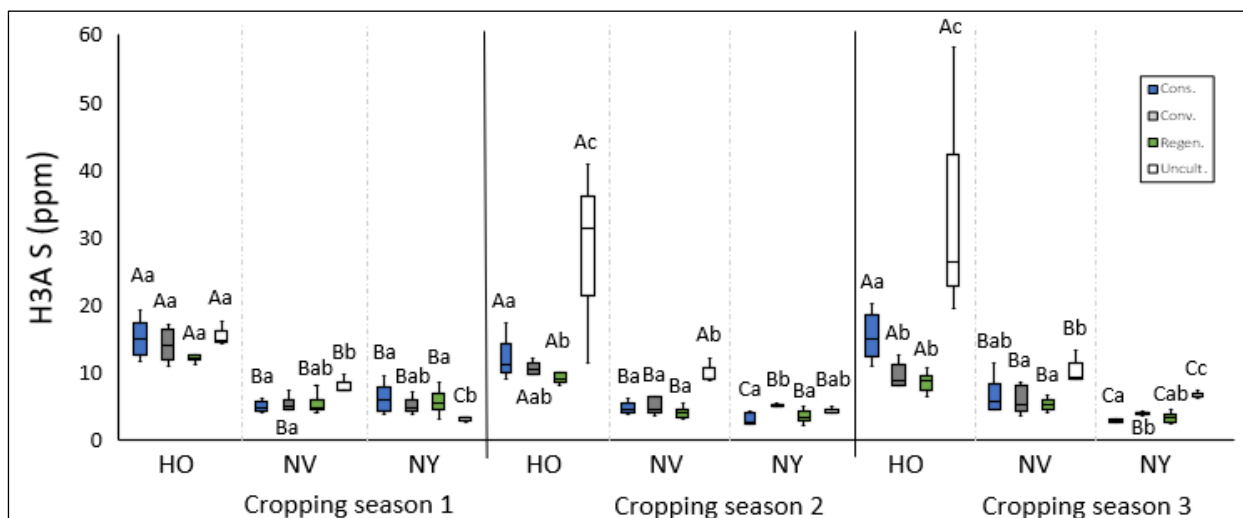


Figure 4.2.9: Pairwise comparisons ($p < 0.05$) of H3A sulphur (S) (parts per million; ppm) across cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$) differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively.

Sodium indicated variability between ecotopes during the first two cropping seasons. Whereby HO (45 ± 76 ppm) significantly differed from both NV (8 ± 5 ppm) and NY (12 ± 1.2 ppm), and the latter did also from one another. This trend was the same during the second cropping season but changed during the final cropping season when NV and NY were no longer significantly different.

The only significant difference between cropping systems during the first cropping season were the Uncult (224 ± 144 ppm) at HO from the remaining implemented systems. Though the Uncult systems all significantly differed from the implemented farming system during the second cropping season, it was additionally noted that Regen (20 ± 4 ppm) system significantly differed from the Conv (20 ± 2 ppm) system at HO. Apart from the significant difference between the Uncult systems and the implemented farming systems, there were no significant differences during the final cropping season at any of the ecotopes.

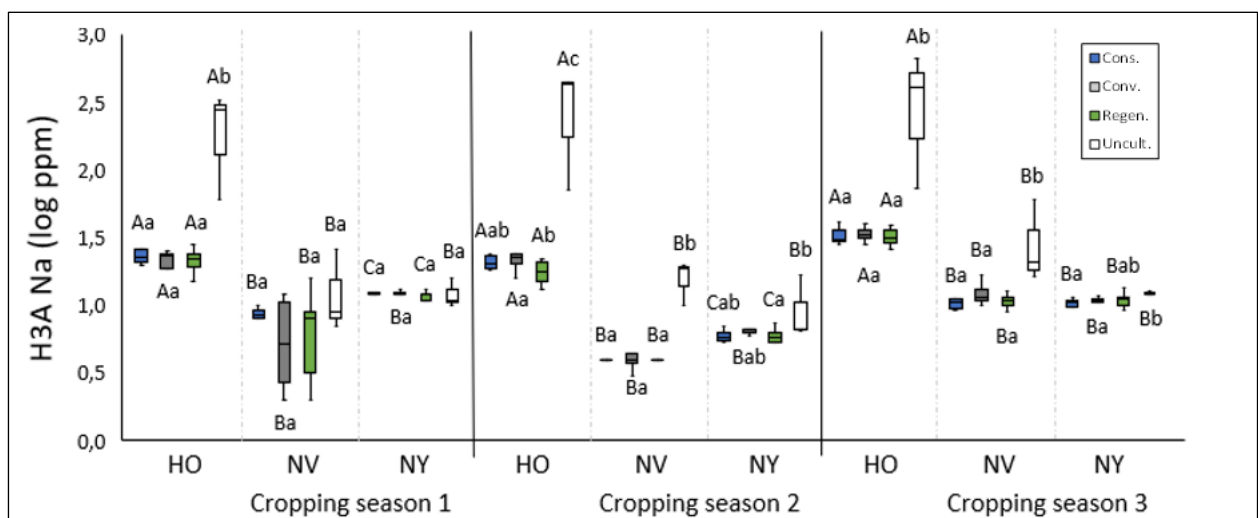


Figure 4.2.10: Pairwise comparisons ($p < 0.05$) of H3A sodium (Na) (displayed as parts per million; ppm) across cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult.) system. Capital and lower-case letters denote significant ($p < 0.05$) differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively.

Inorganic N (Figure 4.2.11) parameter indicated that during the first cropping season, the only significant difference between HO and NV was observed in the Conv systems, with values of 14.2 ± 3.3 ppm and 11.1 ± 1.8 ppm, respectively. The only significant difference between HO and NY was found in the Regen systems, with values of 14.1 ± 5.5 ppm and 20.6 ± 9.1 ppm, respectively. Additionally, NV (12.2 ± 3.3 ppm) and NY (18.4 ± 8.3 ppm) showed significant differences, except for the Uncult system. At HO, the only significant difference between farming systems was between the Uncult system (4.7 ± 2.8 ppm) and the implemented systems (Conv - 14.2 ± 3.3 ppm, Cons - 13.4 ± 3.5 ppm, Regen - 14.1 ± 5.5 ppm). There were no significant differences between farming systems at NV, while at NY, only the Uncult system significantly differed from the implemented systems. During the second cropping season only the Regen and Uncult systems significantly differed between HO and NV, with values of 5.9 ± 1.8 ppm and 6.4 ± 1.1 ppm for Regen, and 8.9 ± 3.3 ppm and 10.7 ± 1.1 ppm for Uncult, respectively. The same significant differences were observed between HO and NY, resulting in no significant differences

between NV and NY. No significant differences were noted between farming systems at HO. However, at NV, the Uncult system significantly differed from both the Cons and Regen systems, which also differed from each other. At NY, the only significant difference was the Cons system (7.6 ± 1.6 ppm) compared to the other farming systems. In the final cropping season, the Cons and Regen systems significantly differed between HO and NV, with values of 11.7 ± 3.7 ppm and 9.1 ± 5.5 ppm for Cons, and 10.2 ± 2.3 ppm and 11.5 ± 4.0 ppm for Regen, respectively. Between HO and NY, the only significant differences were in the Cons and Uncult systems. NV and NY also showed significant differences, except in the Conv system. At HO, the Uncult system significantly differed from the implemented systems, and the Cons system significantly differed from both the Conv and Regen systems. At NV, the only significant difference was between the Uncult system and the Cons and Regen systems. At NY, the Uncult system significantly differed from the implemented systems, and the Cons and Conv systems also significantly differed from each other.

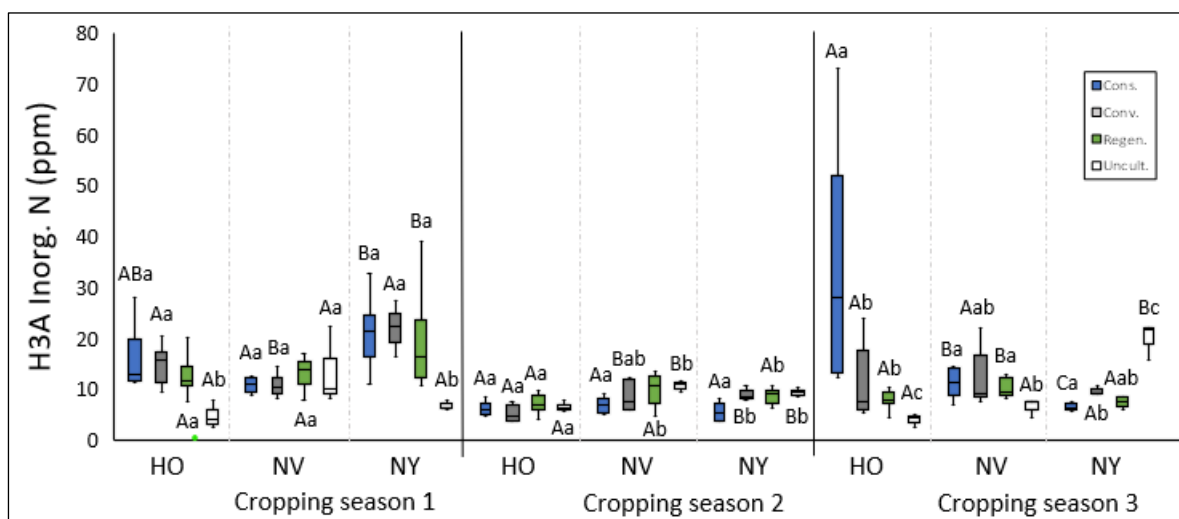


Figure 4.2.11: Pairwise comparisons ($p < 0.05$) of H3A inorganic nitrogen (Inorg. N) (parts per million; ppm) across cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$) differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively.

4.2.3. Biological parameters

The main biological soil health parameter across all three cropping seasons was soil organic matter (%). The pairwise comparison (Figure 4.2.4) revealed that HO ($4 \pm 1\%$) significantly differed from NV ($2 \pm 1\%$) (except for the Uncult system) and from NY ($1 \pm 0.1\%$) during the first cropping season. Additionally, NV and NY were significantly different from each other. The only

significant difference between farming systems was found at HO, where the Uncult system ($7 \pm 2\%$) significantly differed from the implemented systems (Conv – $4 \pm 0.1\%$, Cons – $4 \pm 0.1\%$, Regen – $4 \pm 0.1\%$). The same was observed at NV. At NY, only the Uncult system ($1 \pm 0.1\%$) significantly differed from the Regen system ($0.7 \pm 0.1\%$). In the second cropping season, the significant differences between ecotopes remained the same as in the first cropping season, as did the significant differences between systems at HO and NV. At NY, the Uncult system ($2 \pm 0.4\%$) significantly differed from both the Conv ($0.6 \pm 0.1\%$) and Regen ($0.7 \pm 0.1\%$) systems, with the Conv and Regen systems also significantly differing from each other. In the final cropping season, all significant differences between ecotopes and farming systems were the same as the second cropping season.

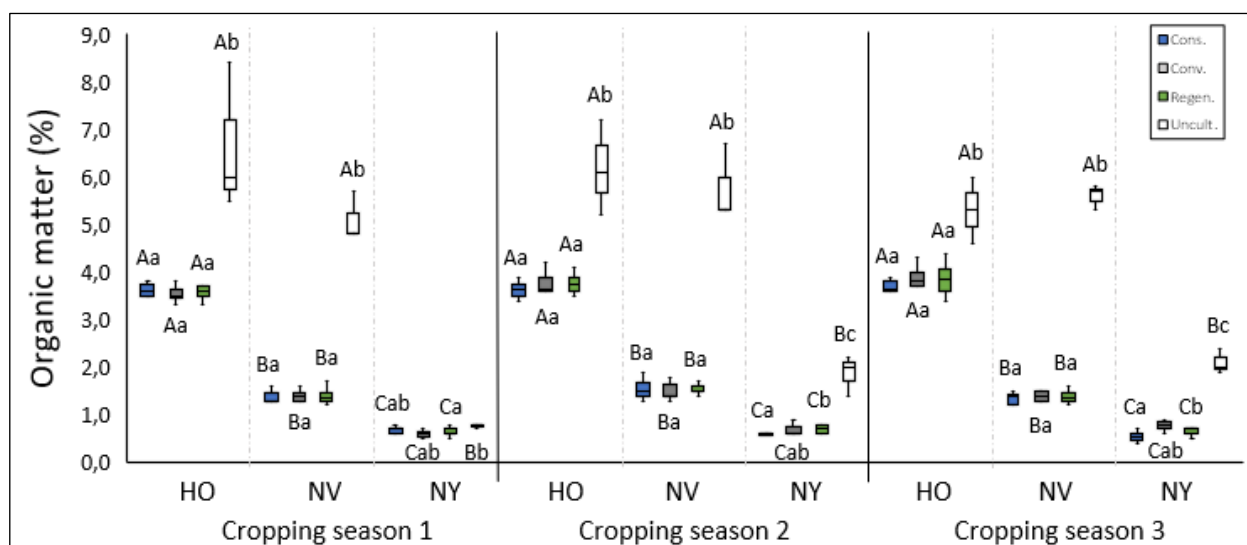


Figure 4.2.12: Pairwise comparisons ($p < 0.05$) of organic matter (%) across cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$) differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively.

4.3. COMPARISON OF SOIL HEALTH SCORES AND IDENTIFICATION OF PROXY INDICATORS

This section uses pairwise comparisons to examine significant differences in soil health scores (Haney analysis) comparing ecotopes and systems per cropping season. A distance-based linear model identified parameters with significant correlations ($r \geq 0.7$; $p < 0.05$), and the top five were ranked by their contribution to soil health, noting that removing the top parameter increased the contribution of the others.

4.3.1. Soil Health Score

Soil health scores followed a similar trend during each of the cropping seasons (Figure 4.3.1). In the first cropping season, HO (14 ± 9), NV (5 ± 3), and NY (3 ± 1) significantly differed from each other at most of the farming systems. The only significant differences between farming systems were observed at HO, where the Uncult system (38 ± 5) significantly differed from the implemented farming systems (Conv – 12 ± 2 , Cons – 10 ± 1 , Regen – 11 ± 2). At NV, the Uncult system (12 ± 0.4) significantly differed from the implemented systems (Conv – 4 ± 1 , Cons – 4 ± 1 , Regen – 4 ± 1). During the second cropping season, all the implemented farming systems from HO significantly differed from NV and NY, while NV and NY did not significantly differ from each other. In the final cropping season, the pattern of significant differences between ecotopes mirrored that of the second season. The significant differences between farming systems at HO and NV remained unchanged. However, at NY, the Cons (4 ± 1) and Regen (4 ± 1) systems significantly differed from the Conv (3 ± 0.4) system, and the Uncult system (5 ± 0.3) significantly differed from all the implemented systems.

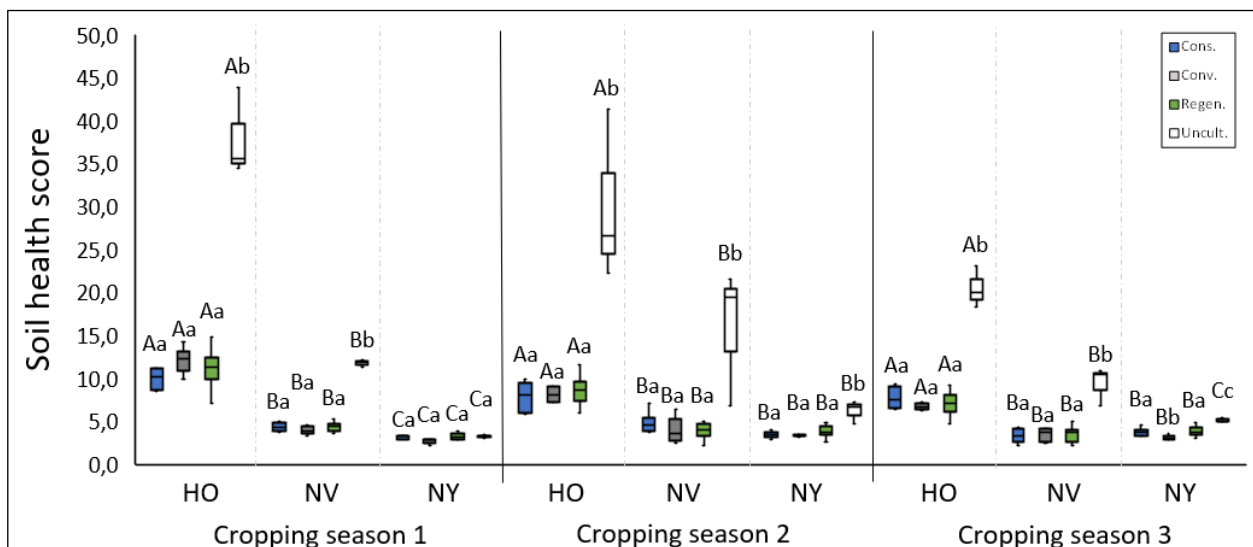


Figure 4.3.1: Pairwise comparisons ($p < 0.05$) of soil health scores across cropping seasons, ecotopes, and farming systems. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY), while coloured bars depict the conventional (Conv), conservation (Cons), and regenerative (Regen) systems, as well as the uncultivated veld (Uncult) system. Capital and lower-case letters denote significant ($p < 0.05$) differences between ecotopes (per cropping season) and farming systems (per ecotope per cropping season), respectively.

4.3.2. Proxy indicators of soil health

All the physical, chemical, and biological soil health parameters (not just the ones that were identified as main parameters) were tested as predictor variables in a distance-based linear model

to the Soil Health Score. Avoiding redundancy, parameters used to compose the soil health score (water extractable organic carbon - WEOC; water extractable organic nitrogen - WEON, and carbon dioxide respiration) were excluded from the analysis. The top five significant ($p < 0.05$) soil health parameters were ranked according to each's proportional contribution to variation in soil health scores. All the systems were taken into consideration, and results are reported per cropping season per ecotope.

At HO, Mg was identified as a significant indicator during the first (77.8%) and third cropping season (7.3%). Organic matter was also a significant indicator across all three cropping seasons, accounting for 8.5% in the first cropping season, 79% in the second, and 2.8% in the final cropping season. Additionally, in the third cropping season, pH became a significant indicator, representing 78.4% of the soil health score.

At NV, OM was the significant indicator during the first cropping season, representing 88% to the soil health score. In the second cropping season, Na emerged as a main indicator, accounting for 67.5%, with total N following at 19.4%. Inorganic N was present in all the cropping seasons, peaking in the third cropping season where it represented 9.4%. By the third cropping season, OM remained the significant indicator, representing 75.8% of the soil health score, highlighting its consistent importance in this ecotope.

At NY, OM was a significant indicator during the second and third cropping season, with the highest contribution during the third cropping season (81.7%). Potassium was notably significant during the first cropping season, representing 42.3% of the soil health score. Microbial active carbon became more prominent during the second and third cropping season, accounting for 4.5% and 10%, respectively. Total N and inorganic N were also recurrent indicators, especially in the first and second cropping seasons.

Table 5: Top five significant soil health parameters ranked according to the proportional contribution to the variation in the soil health score. All systems were taken into consideration, and results are portrayed for per ecotope (Hendrik Odendal – HO, Nicol de Vos – NV, Nant Yzel – NY) per cropping season.

Cropping season 1 - HO		Cropping season 1 - NV		Cropping season 1 - NY	
Variable	Proportion (%)	Variable	Proportion (%)	Variable	Proportion (%)
Mg	77.8	OM	88.0	K	42.3
OM	8.5	Inorganic N	5.1	Inorganic N	22.7
Clay	4.3	Total available N	5.0	Total available N	10.2
Ca	3.3	Organic C:N	0.9	Total N	7.5
Organic C:N	2.5	MAC	0.7	Sand	3.9
Cropping season 2 - HO		Cropping season 2 - NV		Cropping season 2 - NY	
Variable	Proportion (%)	Variable	Proportion (%)	Variable	Proportion (%)

OM	79.1	Na	67.5	OM	76.8
MAC	7.0	Total N	19.4	Organic C:N	10.5
Na	3.7	Inorganic N	5.6	MAC	4.5
Inorganic N	3.1	Fe	1.8	Inorganic N	3.1
Mn	2.9	MAC	1.2	Total available N	2.1
Cropping season 3 - HO		Cropping season 3 - NV		Cropping season 3 - NY	
Variable	Proportion (%)	Variable	Proportion (%)	Variable	Proportion (%)
pH	78.4	OM	75.8	OM	81.7
Mg	7.3	Inorganic N	9.4	MAC	10.0
VAS	3.4	Na	3.8	Total N	3.4
OM	2.8	Organic C:N	2.9	Mn	1.8
MAC	1.3	MAC	2.8	Inorganic N	1.2

* Organic matter (OM); sodium (Na); microbially active carbon (MAC); carbon (C); nitrogen (N); iron (Fe); manganese (Mn); magnesium (Mg); volumetric aggregate stability (VAS)

Overall, OM emerged as the most consistent significant soil health parameter across all ecotopes and cropping seasons. Inorganic N, MAC, and various nutrients such as Mg, Na, and K showed varying levels of proportional contribution to soil health scores depending on the specific ecotope and cropping season.

CHAPTER 5 DISCUSSION

5.1. OVERVIEW OF KEY FINDINGS PREVALENT IN THIS STUDY

The objectives of this study were to 1) characterise soil health properties under conventional, conservation, and regenerative farming systems, 2) evaluate and compare soil health status across cropping seasons (2022/2021, 2021/2022, and 2022/2023), ecotopes (Hendrik Odendal – HO, Nicol de Vos – NV, and Nant Yzel – NY), and farming systems, and 3) identify cost-effective, singular indicators that could serve as proxies for soil health status. To achieve these objectives, physical, chemical, and biological soil health parameters were assessed from which selected parameters were identified as main parameters. Among the physical soil health parameters, sand content, clay content, and volumetric aggregate stability emerged as main parameters across all three cropping seasons. Chemical soil health parameters varied by season: copper (Cu), magnesium (Mg), iron (Fe), and pH were most significant in the first cropping season, sulphur (S), manganese (Mn), and pH in the second, and Fe, sodium (Na), and inorganic nitrogen (N) in the third. Organic matter (OM) consistently emerged as the main biological parameter across all seasons. The soil health score derived from the Haney Analysis highlighted significant differences between ecotopes during each cropping season, but little to no significant differences between farming systems within individual ecotopes. Notably, OM proved to be the most consistent and reliable proxy indicator of soil health, offering a cost-effective measure for evaluating overall soil status.

5.2. IMPACT OF ECOTOPES ON SOIL HEALTH

Ecotopes are characterised by inherent soil health properties (physical, chemical, and biological) that should be considered when evaluating the influence of conservation and regenerative agriculture farming systems on soil health (Amsili et al. 2021; Brady & Weil, 2017; Hensley et al. 2019). Understanding inherent soil health properties is essential for effective land resource planning and sustainable resource utilisation (Maguza et al. 2025; Zuber et al. 2020). The assessment of soil texture as a physical parameter of soil health in this study revealed that NY had the highest sand content and HO the lowest across all three cropping seasons. Conversely, clay content was highest at HO and lowest at NY. This consistent pattern supports the concept of inherent soil properties, as Zaaboubi et al. (2020) note that soil texture is largely unaffected by human intervention. Nonxuba et al. (2025) contribute to this through a study aimed to evaluate the medium-term effects (over ten years) of conservation agricultural practices on selected soil physical properties in the sub-humid region of the Eastern Cape, South Africa. The research aimed to determine how these farming practices influence bulk density, porosity, aggregate

stability, and water retention. The study confirmed that soil texture remained unchanged despite different farming practices, while bulk density, porosity, and water retention showed significant variations due to the implementation of conservation agriculture. Nonxuba et al. (2025) concluded that factors like soil organic matter accumulation, residue retention, and crop rotation influenced soil structure and stability but not sand, silt, and clay composition.

The assessment of chemical parameters in this study revealed that the two main chemical parameters, Mg and Na, exhibited significant differences between ecotopes, with the highest values recorded at HO and the lowest at NY. However, these base cations did not show clear significant differences between the implemented farming systems throughout this study. While it is known that base cations can be influenced by agricultural practices such as fertilisation and liming (Brady & Weil, 2017), the observed relationship between higher concentrations of base cations in ecotopes with finer-textured soils, coupled with the absence of significant differences between farming systems, suggests that Mg and Na as part of the soil's cation exchange capacity are more reflective of inherent soil properties (Sithole et al. 2016). Zuber et al. (2020) confirm this through a study on the role of inherent soil characteristics in assessing soil health across Missouri. Their study concluded that the soil's cation exchange capacity (CEC) followed the same general trend of clay content, due to the small particle size and large surface area of clay particles, which provides more sites for adsorption of cations. The CEC in their study further indicate correlation with organic matter. While Mg and Na were directly linked to inherent soil properties, the co-correlation of K with these base cations in this study suggests that K would have exhibited similar comparative trends and may also serve as an indicator of inherent soil health properties.

Zuber et al. (2020) identified soil organic carbon (SOC), active carbon (AC), and potentially mineralisable nitrogen (PMN) as inherent soil health indicators, recognising their direct relationship with OM content. In this study, OM content varied significantly between ecotopes, suggesting its contribution to parameters that should be considered inherent soil health properties. Although OM and its correlated parameters, as proposed by Zuber et al. (2020), may be regarded as biological indicators of inherent soil health, these properties are more variable and subject to change over time (Bünemann et al. 2018; Veum et al. 2014). This highlights the need to review how the implemented farming systems influenced OM across all cropping seasons.

Soil health properties are interconnected, meaning that no single physical, chemical, or biological parameter can fully define inherent soil health. Instead, each ecotope exhibits a distinct soil health profile shaped by the interaction of these factors (Brady & Weil, 2017; Haney et al. 2018; Zuber et al. 2020). In this study, HO had a higher clay content, which likely contributed to greater surface area for OM retention (Zuber et al. 2020). This, in turn, influenced co-correlated parameters such

as soil respiration and organic C, resulting in significantly higher soil health scores in HO, as determined by the Haney analysis (Haney et al. 2018). Notably, in review of the uncultivated systems, HO exhibited higher soil health scores compared to other ecotopes, further reinforcing the role of inherent soil properties in shaping overall soil health.

5.3. IMPACT OF IMPLEMENTED FARMING SYSTEMS ON SOIL HEALTH

Conventional agriculture can lead to soil degradation, environmental pollution, and biodiversity loss (Lal, 2009; Sanaullah et al. 2020; Savci, 2012; Sithole et al. 2016). In contrast, conservation and regenerative agriculture have showed potential in increasing soil health through soil organic matter retention, enhanced microbial activity, improved soil structure, nutrient cycling and fertility enhancement (Hiel et al. 2016; Nonxuba et al. 2025; Six et al. 2000; Verhulst et al. 2010). Comparing all three implemented farming systems against the uncultivated systems revealed significant differences, indicating to some extent an impact on soil health. It was expected that the conventional systems would present more degraded soils with significant differences between the conventional, conservation, and regenerative farming systems. However, comparisons between these three systems revealed minimal differences. Furthermore, there was no clear indication that conservation and regenerative agriculture leads to significantly different soil health outcomes compared to conventional agriculture in the short term (< 5 years).

Analysis of sand content across cropping seasons within each ecotope revealed no significant differences between the implemented farming systems. Similarly, clay content followed the same pattern, showing no significant difference between implemented farming systems. These findings align with previous studies that demonstrated soil texture is not easily altered by farming practices (Nonxuba et al. 2025; Zaaboubi et al. 2020). Volumetric aggregate stability (VAS) exhibited a single significant difference during the first cropping season at NY, where the regenerative system differed significantly from both the conventional and conservation systems. However, no significant differences were observed in the second and third cropping seasons. This finding contrasts with Jephita et al. (2023), who reported that water-stable aggregates were significantly enhanced by maize-soybean rotations compared to continuous maize systems. Their study demonstrated that crop rotation consistently improved the proportion of water-stable aggregates across sites, highlighting the critical role of legumes like soybeans in promoting soil aggregation. Jephita et al. (2023) further noted that soil organic matter accumulation was a key factor driving these improvements (Colunga et al. 2025; Verhulst et al. 2010). Reduced tillage practices in their study (Jephita et al. 2023) resulted in higher OM levels, which in turn increased the proportion of water-stable aggregates. The reason why this study may differ from these reports lie in the limited

accumulation of soil organic matter. As Strauss et al. (2021a) indicates that soil organic matter accumulation often requires 5-10 years after initiating conservation and regenerative agriculture farming practices. Whereas this study was conducted across three cropping seasons.

Inorganic N, Mg, and S were the only macronutrients to be identified as main parameters during this study. Notably, N content in conservation and regenerative agriculture systems at NY was lower than in conventional systems, particularly during the second and third cropping seasons. This reduction is likely due to the decreased fertiliser application rates at NY, as conservation and regenerative systems utilised less fertiliser compared to the other ecotopes. Additionally, this could be attributed to increased N immobilisation by soil microbes, driven by the high carbon-to-nitrogen (C/N) ratio of crop residues (Jat et al. 2023). It is expected that this finding would have changed with a longer implementation period of crop diversification and rotation that includes legumes since these crops have a greater capacity to fix N in the soil compared to other crops (Nel, 2005). Magnesium showed a significant difference only between the conservation agriculture system and the other implemented systems at NY during the final cropping season. This variation may be attributed to a slight increase in OM at NY. As organic matter possesses the ability to enhance CEC due to a net negative charge that attracts and hold positively charge cations like Mg. Organic matter also provides a larger surface area for cation adsorption (Zuber et al. 2020). The accumulation of OM may also explain the significant differences observed in sulphur (S) content between the conservation and regenerative agriculture systems compared to the conventional systems (Jat et al. 2023; Nonxuba et al. 2025). Potassium (K) was identified as additional parameter co-correlating with organic C, and organic C had a strong positive correlation with OM, suggesting that an increase in OM generally leads to higher K levels (Nonxuba et al. 2025). No clear trends were observed for the micronutrients Fe, Cu, and Mn, which emerged as main parameters under the implementation of conservation and regenerative agriculture in this study. Additionally, Zn, which co-correlated with these micronutrients, was not expected to indicate any clear trends. This contrasts with findings by Turmel et al. (2015) and Jat et al. (2023), who explain that conservation and regenerative agriculture systems generally lead to higher concentrations of micronutrient (as well as macronutrients) in the surface soil layer (0–15 cm) compared to conventional agriculture systems. Furthermore, the only significant difference in pH was at NY during the first cropping season with the regenerative agriculture system being significantly higher than the rest of the implemented systems. Further aligning with the findings of Jat et al. (2023) which stated the effects of conservation and regenerative agriculture on the changes in soil pH are minimal. This might be attributed to a soil's buffer capacity, which is the ability to withstand changes in pH commonly determined by the presence of clay and OM (Brady & Weil, 2017).

Conventional, conservation, and regenerative agricultural practices impact the biological properties of soil health (Perret & Payen, 2020; Sithole et al. 2016). Research indicates that conventional agriculture, with its reliance on intensive tillage, exposes OM to oxidation (Du Preez & Van Huyssteen, 2020; Hobbs, 2007), leading to lower OM levels compared to conservation and regenerative agriculture. The latter approaches promote OM accumulation through practices like permanent soil cover and crop diversification (Hobbs, 2007; Sithole et al. 2016; Turmel et al. 2015). During the first cropping season, no significant differences in OM content were observed between the farming systems across all ecotopes. However, from the second cropping season onward, the regenerative system at NY showed a significant increase in OM compared to the conservation system. Additionally, although not main parameters, organic C, organic N and soil respiration exhibited a strong positive correlation with OM, confirming that the observed co-correlated values could be aligned with the trends in OM (Adetunji et al. 2020). The observed outcome could be attributed to management practices. While conservation agriculture retained permanent soil cover, regenerative agriculture introduced cover crops, which enhanced OM accumulation (Koudahe et al. 2022). These findings align with Dube et al. (2012), who noted that the introduction of cover crops, particularly when paired with low levels of N fertiliser and winter cover crops, leads to the highest OM accumulation in soils.

5.4. IMPACT OF CROPPING SEASON ON SOIL HEALTH

Soil texture, identified as a main physical soil health parameter in this study, remained stable across all three cropping seasons. This was consistent with previous research indicating minimal changes in soil texture during short-term trials (Nonxuba et al. 2025; Zaaboubi et al. 2020). Significant differences in chemical parameters were attributed to the impact of implemented farming systems (Jat et al. 2023; Nonxuba et al. 2025; Zuber et al. 2020). While the absence of significant differences may be attributed to the study's short duration (Swanepoel et al. 2018). Furthermore, the stability of OM across all three seasons can be explained by the extended time required for its accumulation (Strauss et al. 2021a). In essence, Swanepoel et al. (2018) states that the expected effects of conservation and regenerative agriculture in their study did not reflect in the short – or medium – term (< 5 years). This could be because practices associated with conservation and regenerative agriculture (e.g., minimal soil disturbance, permanent soil cover, cover cropping) require time to build up organic matter and microbial biomass. In association with this it was recorded that SOC accumulation often takes 5–10 years to show measurable increases (Colunga et al. 2025). Therefore, longer-term trials are essential to gather the data needed to better understand the impact of conservation and regenerative agriculture on soil health. Farmers

adopting conservation or regenerative agriculture should expect gradual improvements rather than immediate yield increases.

5.5. SOIL HEALTH STATUS UNDER CONSERVATION AND REGENERATIVE AGRICULTURE

Conservation and regenerative agriculture have gained increasing attention in recent years due to its potential to enhance soil health (Dube et al. 2012; Sithole et al. 2016; Swanepoel et al. 2018). Tools like the Haney Analysis have been developed to provide a quantitative value (soil health score) using a set of indicators (soil respiration, water extractable organic N, and water extractable organic C) (Haney et al. 2018). In this study, the only significant improvement in soil health under conservation and regenerative agriculture, as presented by the soil health score, was observed at NY during the third cropping season when compared to conventional agriculture. As the parameters used in calculating the soil health score (Haney et al. 2018) correlate with OM (Adetunji et al. 2020), this observation could be attributed to the impact of farming systems. Specifically, where the only significant improvement of OM content was noted at NY.

5.6. USING BEST SUBSET (PROXY) INDICATORS IN SOIL HEALTH ASSESSMENT

This study identified Mg, OM, pH, Na, K, microbial active carbon (MAC), and N (total and inorganic) as key indicators that can serve as proxies of soil health status (Haney et al. 2018). However, it has been revealed that the effects of conservation and regenerative agriculture on the changes in soil pH are minimal (Jat et al. 2023). Additionally, that Mg, Na, and K are properties of inherent soil health (Sithole et al. 2016; Zuber et al. 2020). Therefore, N, OM, and MAC are the remaining proxies that should be considered as key indicators of change in soil health status. Nitrogen content was influenced by the implemented farming systems (Jat et al. 2023). The initial lower N content observed may be due to increased N immobilisation by soil microbes, driven by the high carbon-to-nitrogen (C/N) ratio of crop residues. This suggests that if N levels decrease during the initial adoption of conservation and regenerative agriculture, farmers might expect an increase in soil microbes. However, in the long term, N content is likely to gradually rise as crop diversification and rotation including leguminous crops enhance N fixation in the soil (Nel, 2005). Microbially active carbon, is a readily available energy source for soil microbes that reflects a soil's biological activity and its ability to support nutrient cycling, which are important component of OM (Brady & Weil, 2017). Additionally, OM correlates with other soil health parameters such as SOC (Zuber et al. 2020). Whereby the amount, quality, and turnover of SOC is vital to soil health because it influences soil structure and aggregation, soil water retention, nutrient retention,

nutrient transformations, and gaseous emissions (Lal, 2016). According to Adetunji et al. (2020), the parameters used in the calculation of the soil health score correlate with OM. Therefore, it is recommended that it is only required to measure N content and OM as proxies of change in soil health status under the implementation of conservation and regenerative agriculture farming systems. However, it is further recommended that all the identified proxies be measured to understand the inherent soil health status prior to implementation of conservation and regenerative agriculture farming systems.

CHAPTER 6 CONCLUSION

6.1. TESTING OF HYPOTHESES

This study aimed to assess the impact of conservation and regenerative agriculture on soil health across multiple ecotopes in maize production regions of South Africa and to identify cost-effective indicators of soil health status. It set out to determine the impact of implemented systems by focusing on the analysis of physical, chemical, and biological parameters representative of soil health. Through completing the objectives, the aims of the study were achieved, and the hypotheses were tested. As a summary, the hypotheses are stated and concluded below:

1. Physical, chemical, and biological properties associated with soil health will vary significantly between conventional, conservation, and regenerative agricultural systems across multiple ecotopes, with regenerative systems expected to exhibit better overall soil health.

The first hypothesis is partially accepted. Although conservation and regenerative farming systems were anticipated to positively influence soil health compared to conventional methods (Hobbs 2007; Sithole et al. 2016; Sumberg & Giller 2022), the findings based on the measured soil health parameters of this study indicate that their impact was minimal over the three cropping seasons. Among the physical soil health properties, it was concluded that soil texture is not easily altered by farming practices (Nonxuba et al. 2025; Zaaboubi et al. 2020). Furthermore, that improved volumetric aggregates stability (VAS) requires accumulation of organic matter (OM) (Jephitha et al. 2023), however, that this is only to be expected after 5-10 years after initiating conservation and regenerative agriculture farming practices (Strauss et al. 2021a). The chemical soil health properties revealed that inorganic nitrogen (inorganic N) could have been influenced by the reduction of fertiliser application and increased N immobilisation by soil microbes because of a high carbon-to-nitrogen (C/N) ratio of crop residues (Jat et al. 2023). No clear trends were observed for the micronutrients Fe, Cu, and Mn. This contrasted observations made by Turmel et al. (2015) and Jat et al. (2023) that suggested conservation and regenerative agriculture systems generally lead to higher concentrations of micronutrients (as well as macronutrients) in the surface soil layer (0–15 cm) compared to conventional agriculture systems. Magnesium (Mg) as a base cation showed a significant difference only between the conservation agriculture system and the other implemented systems at NY during the final cropping season, likely attributed to the correlation with and slight increase in OM (Zuber et al. 2020). This study further confirmed that effects of conservation and regenerative agriculture on the changes in soil pH are minimal (Jat et al. 2023). Biological parameters indicated that from the second cropping season onward, the regenerative system at NY significantly increased OM compared to the conservation system (Dube et al. 2012).

It is noteworthy that even though significant differences were limited, the Nant Yzel ecotope exhibited the most pronounced variations in soil health parameters. Additionally, soil health scores associated with conservation and regenerative agriculture at Nant Yzel showed a significant improvement during the final cropping season, compared to conventional agriculture. This may be attributed to the ecotope's initially low soil health status (Amsili et al. 2021; Brady & Weil, 2017; Hensley et al. 2019), which created a higher potential for measurable improvement compared to the other ecotopes (Zuber et al. 2020).

2. Using soil health scores derived from the Haney analyses, we further hypothesise that specific proxy indicators, such as the macro – and micronutrients, as well as soil organic matter, will emerge as key subsets of soil health status, offering practical, singular metrics for assessing soil health across diverse agricultural systems.

The second hypothesis is accepted. Magnesium (Mg), sodium (Na), potassium (K), microbial active carbon (MAC), nitrogen (both total and inorganic forms), pH, and organic matter (OM) were identified as proxy indicators of soil health in this study. These parameters exhibited the strongest correlations with the Haney soil health score (Haney et al. 2018), demonstrating their potential as reliable indicators for assessing soil health across different agricultural systems (Fine et al. 2017). Notably, their significance varied across different ecotopes and cropping seasons, suggesting that soil health assessments should consider regional variability and temporal fluctuations. Among these indicators, it was recommended that only N content and OM be measured as proxies of change in soil health status under the implementation of conservation and regenerative agriculture farming systems. However, it was further recommended that all the identified proxies be measured to understand the inherent soil health status prior to implementation of conservation and regenerative agriculture farming systems. These findings suggest that a simplified, yet effective soil health assessment framework could be developed using these proxy indicators, potentially offering a more cost-effective alternative to the comprehensive but resource-intensive Haney Soil Health Test.

6.2. FUTURE RECOMMENDATIONS

This study does not provide definitive evidence regarding the ability of conservation and regenerative farming systems to improve soil health compared to conventional methods within the observed timeframe. The findings emphasise the need for long-term studies to assess whether the continued application of these practices can lead to significant improvements in soil health. Further research is required to refine the implementation strategies of conservation and regenerative agriculture principles, particularly in diverse ecotopes and under varying

environmental conditions. There should be an additional focus on validating proxy indicators of soil health over extended periods and across broader geographic regions to ensure their practical applicability for soil health monitoring in South Africa's agricultural landscapes. Additionally, investigating the role of initial soil conditions in shaping agricultural outcomes can be valuable in optimising soil health management strategies.

BIBLIOGRAPHY

Adetunji, A.T., Ncube, B., Mulidzi, R., Lewu, F.B., 2020. Management impact and benefit of cover crops on soil quality: A review. *Soil & Tillage Research*. 204(104717), 2-11.
<https://doi.org/10.1016/j.still.2020.104717>.

Ahmed, N., Zhang, B., Bozdar, B., Chachar, S., Rai, M., Li, J., Li, Y., Hayat, F., Chachar, Z., Tu, P., 2023. The power of magnesium: unlocking the potential for increased yield, quality, and stress tolerance of horticultural crops. *Frontiers in Plant Science*. 14(1), 1-25.
<https://doi.org/10.3389/fpls.2023.1285512>.

Aktar, W., Sengupta, D., Chowdhury, A., 2009. Impact of pesticides use in agriculture: their benefits and hazards. *Interdisc Toxicol*. 2(1), 1–12. <https://doi.org/10.2478/v10102-009-0001-7>.

Amsili, J.P., van Es, H.M., Schindelbeck, R.R., 2021. Cropping system and soil texture shape soil health outcomes and scoring function. *Soil Security*. 4(1), 2-11.
<https://doi.org/10.1016/j.soisec.2021.100012>.

Anderson, M.J., 2017. *Permutational Multivariate Analysis of Variance (PERMANOVA)*. Wiley Stats Reference: Statistics Reference Online.
<https://doi.org/10.1002/9781118445112.stat07841>.

Arias, M.E., Gonzalez-Perez, J.A., Gonzalez-Vila, F.J., Ball, A.S., 2005. Soil health – a new challenge for microbiologists and chemists. *International Microbiology*. 8(1), 13-21.

Asset Research. Participatory systems research on Conservation Agriculture in the Maluti and Mpumalanga Highveld regions. <https://assetresearch.org.za/participatory-systems-research-on-conservation-agriculture-in-the-mpumalanga-highveld-region/> (access 16 October 2022).

Bai, Z., Caspari, T., Gonzalez, M.R., Batjes, N.H., Mäder, P., Bünemann, E.K., de Goede, R., Brussaard, L., Xu, M., Ferreira, C.S.S., Reintam, E., Fan, H., Mihelič, R., Glavan, M., Tóth, Z., 2018. Effects of agricultural management practices on soil quality: A review of long-term experiments for Europe and China. *Agriculture, Ecosystems and Environment*. 265(1), 1-7.
<https://doi.org/10.1016/j.agee.2018.05.028>.

Bekele, D., Birhan, M., 2021. The impact of secondary micronutrients on crop production. *International Journal of Research Studies in Agricultural Sciences*. 7(5), 37-51.
<http://dx.doi.org/10.2139/ssrn.3962862>.

Boj, E., Grané, A., 2024. The robustification of distance-based linear models: Some proposals. *Socio-Economic Planning Sciences*. 95(2024), 2-17.
<https://doi.org/10.1016/j.seps.2024.101992>.

Brady, N.C., Weil, R.R., 2017. *The Nature and Properties of Soil*. Fifteenth ed. Essex, England.

Bünemann, E.K., Bongiorno, G., Bai, Z., Greamer, R.E., De Deyn, G., De Goede, R., Flesskens, L., Geissen, V., Kuyper, T.W., Mäder, P., Pulleman, M., Sukkel, W., Van Groenigen, J.W., Brussaard, L., 2018. Soil quality – A Critical Review. *Soil Biology and Biochemistry*. 120(2018), 105-125. <https://doi.org/10.1016/j.soilbio.2018.01.030>.

Chandini, P., Kumar, R., Kumar, R., Prakash, O., 2019. The Impact of Chemical Fertilisers on Our Environment and Ecosystems, in: Sharma, P. (Eds.), *Research Trends in Environmental Science*. AkiNik Publications., India, pp. 71-85.

Clark, E.A., 2004. Benefits of Re-Integrating Livestock and Forages in Crop Production Systems. *Journal of Crop Improvement*. 12(1-2), 405-436.
https://doi.org/10.1300/J411v12n01_06.

Colunga, S.L., Wahab, L., Fierro-Cabo, A., Pereira, E., 2025. Carbon sequestration through conservation tillage in sandy soils of arid and semi-arid climates: A meta-analysis. *Soil & Tillage Research*. 245(1), 1-13. <https://doi.org/10.1016/j.still.2024.106310>.

Conant, R.T., Easter, M., Paustian, K., Swan, A., Williams, S., 2007. Impacts of periodic tillage on soil C stocks: A synthesis. *Soil & Tillage Research*. 95(1), 1-10.
<https://doi.org/10.1016/j.still.2006.12.006>.

Cong, W-F., Hoffland, E., Li, L., Six, J., Sun, J-H., Bao, X-G, Zhang, F-S., Van Der Werf, W., 2015. Intercropping enhances soil carbon and nitrogen. *Global Change Biology*. 21(4), 1715-1726. <https://doi.org/10.1111/gcb.12738>.

Department of Agriculture, Land Reform and Rural Development (DALRRD) (South Africa). 2021. Summer crops-Final production estimate (2021) and winter cereals-fourth production estimate (2021). <https://www.old.dalrrd.gov.za/Home/Crop-Estimates> (accessed 25 April 2022).

Department of Agriculture, Land Reform and Rural Development (DALRRD) (South Africa). 2022. Revised area and 1st production forecast for summer crops (2022) and final production estimate for winter cereals (2021). <https://www.dalrrd.gov.za/Home/Crop-Estimates> (accessed 25 April 2022).

Degrendele, C., Klánová, J., Prokeš, R., Příbylová, P., Šenk, P., Šudoma, M., Rösli, M., Dalvie, M.A., Fuhmann, S., 2022. Current use pesticides in soil and air from two agricultural sites in South Africa: Implications for environmental fate and human exposure. *Science of the Total Environment*. 807(1), 1-12 <https://doi.org/10.1016/j.scitotenv.2021.150455>.

Doran, J.W., Parkin, T.B., 1994. Defining and assessing soil quality, in: . Doran, J.W., Coleman, D.C., Bezdicek, D.F., Stewart, B.A. (Eds.), *Defining Soil Quality for a Sustainable Environment*. Soil Science Society of America Special Publications no. 35., Wisconsin, United States of America, pp. 3-21.

Doran, J.W., 2002. Soil health and global sustainability: translating science into practice. *Agriculture, Ecosystems & Environment*. 88(2), 119-127. [https://doi.org/10.1016/S0167-8809\(01\)00246-8](https://doi.org/10.1016/S0167-8809(01)00246-8).

Du Preez, C.C., Van Huyssteen, C.W., 2019. Threats to soil and water resources in South Africa. *Environmental Research*. 183(1), 1-16. <https://doi.org/10.1016/j.envres.2019.109015>.

Dube, E., Chiduza, C., Muchaonyerwa, P., 2012. Conservation agriculture effects on soil organic matter on a Haplic Cambisol after four years of maize–oat and maize–grazing vetch rotations in South Africa. *Soil and Tillage Research*. 123(2012), 21-28. <https://doi.org/10.1016/j.still.2012.02.008>.

Food and Agriculture Organization of the United Nation (FAO). *Conservation Agriculture*. <https://www.fao.org/conservation-agriculture/en/> (accessed 28 August 2023).

Findlater, K.M., Kandlikara, B., Satterfield, T., 2019. Misunderstanding conservation agriculture: Challenges in promoting, monitoring, and evaluating sustainable farming. *Environmental Science and Policy*. 100(1), 47-54. <https://doi.org/10.1016/j.envsci.2019.05.027>.

Fine, A.K., Van Es, H.M., Schindelbeck, R.R., 2017. Statistics, Scoring Functions, and Regional Analysis of a Comprehensive Soil Health Database. *Soil Science Society of America Journal*. 81(3), 590-601. <https://doi.org/10.2136/sssaj2016.09.0286>.

García-Palacios, P., Alarcón, M.R., Tenorio, J.L., Moreno, S.S., 2019. Ecological intensification of agriculture in drylands. *Journal of Arid Environments*. 167(1), 101-105. <https://doi.org/10.1016/j.jaridenv.2019.04.014>.

Gaskin, J., Arriaga, F., Franzluebbers, A., Feng, Y., 2020. Benefits of Increasing Soil Organic Matter, in: Bergtold, J. & Sailus, M. (Eds.). *Conservation Tillage Systems in the Southeast: Production, Profitability, and Stewardship*. SARE., USA, pp. 29-47.

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(2018), 162-168. <https://doi.org/10.1016/j.apsoil.2017.07.035>.

Hayat, R., Ali, S., Amara, U., Khalid, R., Ahmed, I., 2010. Soil beneficial bacteria and their role in plant growth promotion: a review. *Annals of Microbiology*. 60(2010), 579-598. <https://doi.org/10.1007/s13213-010-0117-1>.

He, H., Liu, L., Munir, S., Bashir, N.H., Wang, Y., Yang, J., Li, C., 2019. Crop diversity and pest management in sustainable agriculture. *Journal of Integrative Agriculture*. 18(9), 1945–1952. [https://doi.org/10.1016/S2095-3119\(19\)62689-4](https://doi.org/10.1016/S2095-3119(19)62689-4).

Hensley, M., Le Roux, P.A.L., Botha, J.J., Van Rensburg, L.D., 2019. The role of water conservation strategies and benchmark ecotopes for increasing yields in South Africa's semi-arid croplands. *Water South Africa*. 45(3), 393-399. <https://doi.org/10.17159/wsa/2019.v45.i3.6736>.

Hiel, M-P., Chélin, M., Parvin, N., Barbieux, S., Degruene, F., Lemtiri, A., Colinet, G., Degré, A., Bodson, B., Garré, S., 2016. Crop residue management in arable cropping systems under temperate climate. Part 2: Soil physical properties and crop production. *Biotechnologie*,

Agronomie, Societe et Environnement. 20(1), 245-256. <https://doi.org/10.25518/1780-4507.12986>.

Hilimire, K., 2011. Integrated Crop/Livestock Agriculture in the United States: A Review. *Journal of Sustainable Agriculture*. 35(4), 376-393. <https://doi.org/10.1080/10440046.2011.562042>.

Hobbs, P.R., 2007. Paper Presented at International Workshop on Increasing Wheat Yield Potential, CIMMYT, Obregon, Mexico, 20-24 March 2006. Conservation agriculture: What is it and why is it important for future sustainable food production. *Journal of Agricultural Science*. 145(2), 127-137. <https://doi.org/10.1017/S0021859607006892>.

Hong, S-K., Kim, S., Cho, K-H., Kim, J-E., Kang, S., Lee, D., 2004. Ecotope mapping for landscape ecological assessment of habitat and ecosystem. *Ecological Research*. 19(1), 131-139. <https://doi.org/10.1111/j.1440-1703.2003.00603.x>.

Jat, M.L., Gathala, M.K., Choudhary, M., Sharma, S., Jat, H.S., Gupta, N., Singh, Y., 2023. Conservation agriculture for regenerating soil health and climate change mitigation in smallholder systems of South Asia. *Advances in Agronomy*. 181(1), 183-277. <https://doi.org/10.1016/bs.agron.2023.05.003>.

Jayasinghe, S.L., Thomas, D.T., Anderson, J.P., Chen, C., Macdonald, C.T., 2023. Global Application of Regenerative Agriculture: A Review of Definitions and Assessment Approaches. *Sustainability*. 15(1), 2-49. <https://doi.org/10.3390/su152215941>.

Jephita, G., Jefline, K., Willis, G., Justice, N., 2023. Carbon stock, aggregate stability and hydraulic properties of soils under tillage, crop rotation and mineral fertiliser application in sub-humid Zimbabwe. *Heliyon*. 9(5), 2-15. <https://doi.org/10.1016/j.heliyon.2023.e15846>.

Jolliffe, I.T., 2002. *Principle Component Analysis*. second ed. New York, United States of America.

Jones Jr., J.B., 2012. *Plant Nutrition and Soil Fertility Manual*. second ed. Florida, United States of America.

- Jungers, J.M., DeHaan, L.H., Mulla, D.J., Sheaffer, C.C., Wyse, D.L, 2019. Reduced nitrate leaching in a perennial grain crop compared to maize in the Upper Midwest, USA. *Agriculture, Ecosystems & Environment*. 272(2019), 63-73. <https://doi.org/10.1016/j.agee.2018.11.007>.
- Kassam A., Friedrich, T., Derpsch, R., 2019. Global spread of CA. *International Journal of Environmental Studies*. 7(1), 29–51. <https://doi.org/10.1080/00207233.2018.1494927>.
- Knot, J., Trytsman, G., Smith, H., Du Preez, G., 2022. Participatory systems research on Conservation Agriculture in the Maluti and Mpumalanga Highveld regions: Annual Progress Report. Submitted to ASSET Research.
- Koudahe, K., Allen, S.C., Djaman, K., 2022. Critical review of the impact of cover crops on soil properties. *International Soil and Water Conservation Research*. 10(2),1-12. <https://doi.org/10.1016/j.iswcr.2022.03.003>.
- Kumar, S., Kumar, S., Mohapatra, T., 2021. Interaction Between Macro- and Micro-Nutrients in Plants. *Frontiers in Plant Science*. 12(2021), 1-9. <https://doi.org/10.3389/fpls.2021.665583>.
- Laishram, J., Saxena, K.G., Maikhuri, R.K., Rao, K.S., 2012. Soil Quality and Soil Health: A Review. *International Journal of Ecology and Environmental Sciences*. 38(1), 19-37.
- Lal, R., 2004. Soil Carbon Sequestration Impacts on Global Climate Change and Food Security. *Science*. 304(5677), 1623-1627. <https://doi.org/10.1126/science.109739>.
- Lal, R., 2009. Soil degradation as a reason for inadequate human nutrition. *Food Security*. 1(1), 45-57. <https://doi.org/10.1007/s12571-009-0009-z>.
- Lal, R., 2016. Soil health and carbon management. *Food and Energy Security*. 5(4), 1-11. <https://doi.org/10.1002/fes3.96>.
- Lemaire, G., Franzluebbers, A., Carvalho, P.C.de F., Dedieu, B., 2014. Integrated crop–livestock systems: Strategies to achieve synergy between agricultural production and environmental quality. *Agriculture, Ecosystems and Environment*. 190(1), 4-8. <https://doi.org/10.1016/j.agee.2013.08.009>.

Liebenberg, A., van der Nest, J.R., Hardie, A.G., Labuschagne, J., Swanepoel, P.A., 2020. Extent of Soil Acidity in No-Tillage Systems in the Western Cape Province of South Africa. *Land*. 9(361), 1-17. <https://doi.org/10.3390/land9100361>.

Liebig, M.A., Tanaka, D.L., Kronberg, S.L., Scholljegerdes, E.J., Karn, J.F., 2011. Integrated crops and livestock in central North Dakota, USA: Agroecosystem management to buffer soil change. *Renewable Agriculture and Food Systems*. 27(2), 115–124. <https://doi.org/10.1017/S1742170511000172>.

Maitra, S., Hossain, A., Brestic, M., Skalicky, M., Ondrisik, P., Gitari, H., Brahmachari, K., Shankar, T., Bhadra, P., Palai, J.B., Jena, J., Bhattacharya, U., Duvvada, S.K., Lalichetti, S., Sairam, M., 2021. Intercropping - A Low Input Agricultural Strategy for Food and Environmental Security. *Agronomy*. 11(343), 2-28. . <https://doi.org/10.3390/agronomy11020343>.

Malik, A.A., Puissant, J., Buckeridge, K.M., Goodall, T., Jehmlich, N., Chowdhury, S., Gweon, H.S., Peyton, J.M., Mason, K.E., Van Agtmaal, M., Bland, A., Clark, I.M., Whitaker, J., Pywell, R.F., Ostle, N., Gleixner, G., Griffiths, R.I., 2018. Land use driven change in soil pH affects microbial carbon cycling processes. *Nature Communications*. 9(1), 1-10. <https://doi.org/10.1038/s41467-018-05980-1>.

Massah, J., Azadegan, B., 2016. Effects of chemical fertiliser on soil compaction and degradation. *Agricultural Mechanization in Asia, Africa, and Latin America*. 47(1), 44-50.

Maguza, J., Maliondoa, S.M., Ulrikb, I., Katania, J.Z., 2025. Developing soil quality indices for predicting site classes in *Pinus patula* stands of Sao Hill and Shume Forest Plantations, Tanzania. *Sustainable Environment*. 11(1), 1-12. <https://doi.org/10.1080/27658511.2024.2433330>.

Miller, R.O., Gavlak, R., Horneck, D., 2013. Western States Laboratory Soil, Plant, and Water Analysis Manual. Fourth ed. (WREP 125).

Muhammetoglu, A., Durmaz, S., Uslu, B., 2010. Evaluation of the Environmental Impact of Pesticides by Application of Three Risk Indicators. *Environmental Forensics*. 11(1/2), 179-186. <https://doi.org/10.1080/15275920903559180>.

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. *Agricultural Economics Research, Policy and Practice in Southern Africa*. 62(2), 133-151. <https://doi.org/10.1080/03031853.2023.2169481>.

Muñoz-Rojas, M., 2018. Soil quality indicators: critical tools in ecosystem restoration. *Current Opinion in Environmental Science & Health*. 5(1), 47-52. <https://doi.org/10.1016/j.coesh.2018.04.007>.

Muzangwa, L., Mnkeni, P.N.S., 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(1), 66-76. <https://doi.org/10.1007/s40003-019-00402-0>.

Neina, D., 2019. The Role of Soil pH in Plant Nutrition and Soil Remediation. *Applied and Environmental Soil Science*. 2019(1). 1-9. <https://doi.org/10.1155/2019/5794869>.

Nel, A.A., 2005. Crop rotation in the summer rainfall area of South Africa. *South African Journal of Plant and Soil*. 22(4), 274-278. https://doi.org/10.10520/AJA02571862_597.

Newton, P., Civita, N., Frankel-Goldwater, L., Bartel, K., Johns, C., 2020. What Is Regenerative Agriculture? A Review of Scholar and Practitioner Definitions Based on Processes and Outcomes. *Frontiers in Sustainable Food Systems*. 4(577723),1-11. <https://doi.org/103389/fsufs.2020.577723>.

Nguemezi, C., Tematio, P., Yemefack, M., Tsozue, D., Silatsa, T.B.F., 2020. Soil quality and soil fertility status in major soil groups at the Tombel area, South-West Cameroon. *Heliyon*. 6(2), 1-10. <https://doi.org/10.1016/j.heliyon.2020.e03432>.

Nonxuba, C.S., Elephant, D.E., Nciizah, A.D., 2025. Medium-term effects of tillage, crop rotation and crop residue management practices on selected soil physical properties in the sub-humid region of Eastern Cape, South Africa. *Soil and Tillage Research*. 248(1), 2-10. <https://doi.org/10.1016/j.still.2024.106420>.

Oil and Protein seeds development Trust (OPDT) (South Africa). 2024. Crop Estimates. <https://www.opot.co.za/> (accessed 12 January 2025).

Omer, M., Idowu, O.J., Ulery, A.L., van Leeuwen, D., Guldán, S.J., 2018. Seasonal Changes of Soil Quality Indicators in Selected Arid Cropping Systems. *Agriculture*. 8(8), 1-12. <https://doi.org/10.3390/agriculture8080124>.

Ouda, S., Zohry, A.E., Noreldin, T., 2018. *Crop Rotation: An Approach to Secure Future Food*, first ed. Cham, Switzerland.

Palmer, A.R., Ainslie, A.M., 2005. Grasslands of South Africa, in: Suttie, J.M., Reynolds, S.G., Batello, C. (Eds.), *Grasslands of the World. Food and Agriculture of the United Nations.*, Rome, (e-Book).

Payen, S., Basset-Mens, C., Nunez, M., Follain, S., Grunberger, O., Marlet, S., Perret, S., Roux, P., 2016. Salinisation impacts in life cycle assessment: a review of challenges and options towards their consistent integration. *The International Journal of Life Cycle Assessment*. 21(4), 577–594. <https://doi.org/10.1007/s11367-016-1040-x>.

Perret, S.R., Payen, S., 2020. Irrigation and the Environmental Tragedy: Pathways Towards Sustainability in Agricultural Water Use. *Irrigation & Drainage*. 69(2), 263-271. <https://doi.org/10.1002/ird.2404>.

Pingali, P.L., 2012. Green Revolution: Impacts, limits, and the path ahead. *Proceedings of the National Academy of Sciences of the United States of America*. 109(31), 12302–12308. <https://doi.org/10.1073/pnas.0912953109>.

Rengasamy, P., 2006. World salinization with emphasis on Australia. *Journal of Experimental Botany*. 57(5), 1017-1023. <https://doi.org/10.1093/jxb/erj108>.

Rezaei, S.A., Gilkes, R.J., Andrews, S.S., 2006. A minimum data set for assessing soil quality in rangelands. *Geoderma*. 136(1-2), 229-234. <https://doi.org/10.1016/j.geoderma.2006.03.021>.

Rhodes, C.J., 2017. The imperative for regenerative agriculture. *Science Progress*. 100(1), 80-129. <https://doi.org/10.3184/003685017X14876775256165>.

Rieke, E.L., Bagnall, D.K., Morgan, C.L.S., Flynn, K.D., Howe, J.A., Greub, K.L.H., Mac Bean, G., Cappellazzi, S.B., Cope, M., Liptzin, D., Norris, C.E., Tracy, P.W., Aberle, E., Ashworth, A.,

Banuelos Tavaréz, O., Bary, A.I., Baumhardt, R.L., Borbon Gracia, A., Brainard, D.C., Brennan, J.R., Briones Reyes, D., Bruhjell, D., Carlyle, C.N., Crawford, J.J.W., Creech, C.F., Culman, S.W., Deen, B., Dell, C.J., Derner, J.D., Ducey, T.F., Duiker, S.W., Dyck, M.F., Ellert, B.H., Entz, M.H., Espinosa Solorio, A., Fonte, S.J., Fonteyne, S., Fortuna, A-M., Foster, J.L., Fultz, L.M., Gamble, A.V., Geddes, C.M., Griffin-LaHue, D., Grove, J.H., Hamilton, S.K., Hao, X., Hayden, Z.D., Honsdorf, N., Ippolito, J.A., Johnson, G.A., Kautz, M.A., Kitchen, N.R., Kumar, S., Kurtz, K.S.M., Larney, F.J., Lewis, K.L., Liebman, M., Lopez Ramirez, A., Machado, S., Maharjan, B., Martinez Gamino, M.A., May, W.E., McClaran, M.P., McDaniel, M.D., Millar, N., Mitchell, J.P., Moore, A.D., Moore Jr., P.A., Mora Gutierrez, M., Nelson, K.A., Omondi, E.C., Osborne, S.L., Osorio Alcala, L., Owens, P., Pena-Yewtukhiw, E.M., Poffenbarger, H.J., Ponce Lira, B., Reeve, J.R., Reinbott, T.M., Reiter, M.S., Ritchey, E.L., Roozeboom, K.L., Rui, Y., Sadeghpour, A., Sainju, U.M., Sanford, G.R., Schillinger, W.F., Schindelbeck, R.R., Schipanski, M.E., Schlegel, A.J., Scow, K.M., Sherrod, L.A., Shober, A.L., Sidhu, S.S., Solís Moya, E., St. Luce, M., Strock, J.S., Suyker, A.E., Sykes, V.R., Tao, H., Trujillo Camposi, A., Van Eerd, L.L., Van Es, H.M., Verhulst, N., Vyn, T.J., Wang, Y., Watts, D.B., Wright, D.L., Zhang, T., Honeycutt, C.W., 2022. Evaluation of Aggregate Stability Methods for Soil Health. *Geoderma*. 428(1), 2-13. <https://doi.org/10.1016/j.geoderma.2022.116156>.

Sanauallah, M., Usman, M., Wakeel, A., Cheema, S.A., Ashraf, I., Farooq, M., 2020. Terrestrial ecosystem functioning affected by agriculture management systems: A review. *Soil and Tillage Research*. 196(1), 1-11. <https://doi.org/10.1016/j.still.2019.104464>.

Sarkar, D., Kar, S.K., Chattopadhyay, A., Rakshit, A., Tripathi, V.K., Dubey, P.K., Abhilash, P.C., 2020. Low input sustainable agriculture: A viable climate-smart option for boosting food production in a warming world. *Ecological Indicators*. 115(1), 1-13. <https://doi.org/10.1016/j.ecolind.2020.106412>.

Savci, S., 2012. An agricultural pollutant: Chemical fertiliser. *International Journal of Environmental Science and Development*. 3(1), 77-80. <https://doi.org/10.7763/IJESD.2012.V3.191>.

Schindelbeck, R.R., Moebius-Clune, B.N., Moebius-Clune, D.J., Kurtz, K.S., van Es, H.M., 2016. Standard Operating Procedures, Cornell Soil Health Laboratory, February 2016. Cornell University, Ithaca, NY.

Sekaran, U., Lai, K., Ussiri, D.A.N., Kumar, S., Clay, S., 2021. Role of integrated crop-livestock systems in improving agriculture production and addressing food security – A review. *Journal of Agriculture and Food Research*. 5(1), 1-10.

Shahzad, M., Hussain, M., Jabran, K., Farooq, M., Farooq, S., Gašparovic, K., Barboricova, M., Aljuaid, B.S., El-Shehawi, A.M., Zuan, A.T.K., 2021. The Impact of Different Crop Rotations by Weed Management Strategies' Interactions on Weed Infestation and Productivity of Wheat (*Triticum aestivum* L.). *Agronomy*. 11(2088), 1-15. <https://doi.org/10.3390/agronomy11102088>.

Sharma, P., Singh, A., Kahlon, C.S., Brar, A.S., Grover, K.K., Dia, M., Steiner, R.L., 2018. The role of cover crops towards sustainable soil health and agriculture - a review paper. *American Journal of Plant Sciences*. 9(9),1935-1951. <https://doi.org/10.4236/ajps.2018.99140>.

Sheppard, S.C., Addison, J.A., 2008. Soil sample handling and storage, in: Carter, M.R., Gregorich, E.G. (Eds), *Soil Sampling and Methods of Analysis*. second ed. Canadian Society of Soil Science, CRC Press and Taylor & Francis Group. Oxford, UK. pp. 39-49.

Shoji, S., Delgado, J., Mosier, A., Miura, Y., 2001. Use of Controlled Release Fertilisers and Nitrification Inhibitors to Increase Nitrogen Use Efficiency and to Conserve Air and water Quality. *Communications in Soil Science & Plant Analysis*. 32(7/8), 1051-1070. <https://doi.org/10.1081/CSS-100104103>.

Sithole, N.J., Magwaza, L.S., Mafongoya, P.L., 2016. Conservation agriculture and its impact on soil quality and maize yield: A South African perspective. *Soil and Tillage Research*. 162(1), 55-67. <https://doi.org/10.1016/j.still.2016.04.014>.

Six, J., Elliott, E.T., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate formation: A mechanism for C sequestration under no-tillage agriculture. *Soil Biology and Biochemistry*. 32(14), 2099–2103. [https://doi.org/10.1016/S0038-0717\(00\)00179-6](https://doi.org/10.1016/S0038-0717(00)00179-6).

Smit, H.E., Strauss, J.A., Swanepoel, P.A., 2021. Utilisation of cover crops: Implications for conservation agriculture systems in a Mediterranean climate region of South Africa. *Plant and Soil*. 462(1/2), 207-218. <https://doi.org/10.1007/s11104-021-04864-6>.

Steenwerth, K., Belina, K.M., 2008. Cover crops enhance soil organic matter, carbon dynamics and microbiological function in a vineyard agroecosystem. *Applied Soil Ecology*. 40(2), 359-369. <https://doi.org/10.1016/j.apsoil.2008.06.006>.

Strauss, J.A., Swanepoel, P.A., Smith, H., Smit, E.H., 2021a. A history of conservation agriculture in South Africa. *South African Journal of Plant and Soil*. 38(3), 196-201. <https://doi.org/10.1080/02571862.2021.1979112>.

Strauss, J.A., Swanepoel, P.A., Laker, M.C., Smith, H.J., 2021b. Conservation Agriculture in rainfed annual crop production in South Africa. *South African Journal of Plant and Soil*. 38(3), 217-230. <https://doi.org/10.1080/02571862.2021.1891472>.

Sumberg, J., Giller, K.E., 2022. What is 'conventional' agriculture? *Global Food Security*. 32(1), 1-9. <https://doi.org/10.1016/j.gfs.2022.100617>.

Swanepoel, C.M., Rotter, R.P., van der Laan, M., Annandale, J.G., Beukes, D.J., du Preez, C.C., Swanepoel, L.H., van der Merwe, A., Hoffmann, M.P., 2018. The benefits of conservation agriculture on soil organic carbon and yield in southern Africa are site-specific. *Soil & Tillage Research*. 183(1), 72-82. <https://doi.org/10.1016/j.still.2018.05.016>.

Tanveer, M., Ahmad, S., Hussain, S., Cerda, A., Ashraf, U., 2017. Relay cropping as a sustainable approach: problem and opportunities for sustainable crop production. *Environmental Science and Pollution Research*. 24(8), 6973–6988. <https://doi.org/10.1007/s11356-017-8371-4>.

Teague, R., Kreuter, U., 2020. Managing Grazing to Restore Soil Health, Ecosystem Function, and Ecosystem Services. *Frontiers in Sustainable Food Systems*. 4(534187), 2-13. <https://doi.org/10.3389/fsufs.2020.534187>.

Thapa, S., Bhandari, A., Ghimire, R., Xue, Q., Kidwaro, F., Ghatrehsamani, S., Maharjan, B., Goodwin, M., 2021. Managing Micronutrients for Improving Soil Fertility, Health, and Soybean Yield. *Sustainability*. 13(11766), 1-15. <https://doi.org/10.3390/su132111766>.

Turmel, M-S., Speratti, A., Baudron, F., Verhulst, N., Govaerts, B., 2015. Crop residue management and soil health: A systems analysis. *Agricultural Systems*. 134(1),6-16
<https://doi.org/10.1016/j.agsy.2014.05.009>.

Van Antwerp, R., 2005. A review of soil health indicators for laboratory use in the South African sugar industry.
https://www.researchgate.net/publication/228644789_A_review_of_soil_health_indicators_for_laboratory_use_in_the_South_African_sugar_industry (accessed 14 November 2024).

Veum, K.S., Goyne, K.W., Kremer, R.J., Miles, R.J., Sudduth, K.A., 2014. Biological indicators of soil quality and soil organic matter characteristics in an agricultural management continuum. *Biochemistry*. 117(1), 81-99. <https://doi.org/10.1007/s10533-013-9868-7>.

Verhulst, N., Govaerts, B., Verachtert, E., Castellanos-Navarrete, A., Mazzalama, M., Wall, P., Sayre, K. D. 2010. Conservation agriculture, improving soil quality for sustainable production systems, in: Lal, R., Stewart, B. A. (Eds.), *Food security and soil quality*. CRC press., London, pp. 138-186.

Waldner, F., Hansen, M.C., Potapov, P.V., Löw, F., Newby, T., Ferreira, S., Defourny, P., 2017. National-scale cropland mapping based on spectral-temporal features and outdated land cover information. *PLOS ONE*. 12(8),1-24. <https://doi.org/10.1371/journal.pone.0181911>.

Whittaker, R.H., Levin, S.A., Root, R.B., 1973. Niche, Habitat, and Ecotope. *American Society of Naturalists*. 107(955), 321-338.

Yang, T., Siddique, K.H.M., Liu, K., 2020. Cropping systems in agriculture and their impact on soil health-A review. *Global Ecology and Conservation*. 23(1), 1-13.
<https://doi.org/10.1016/j.gecco.2020.e011118>.

Yu, T., Mahe, L., Li, Y., Wei, X., Deng, X., Zhang, D., 2022. Benefits of Crop Rotation on Climate Resilience and Its Prospects in China. *Agronomy*. 12(2), 1-18.
<https://doi.org/10.3390/agronomy12020436>.

Zaaboubi, S., Khiari, L., Abdesselam, S., Gallichand, J., Kebede, F., Kerrache, G., 2020. Particle Size Imbalance Index from Compositional Analysis to Evaluate Cereal Sustainability for

Arid Soils in Eastern Algeria. *Agriculture*. 10(7), 1-10.

<https://doi.org/10.3390/agriculture10070296>.

Zeng, R., Chen, L., Wang, X., Cao, J., Li, X., Xu, X., Xia, Q., Chen, T., Zhang, L., 2020. Effect of Waterlogging Stress on Dry Matter Accumulation, Photosynthesis Characteristics, Yield, and Yield Components in Three Different Ecotypes of Peanut (*Arachis hypogaea* L.). *Agronomy*. 10(9), 1-15. <https://doi.org/10.3390/agronomy10091244>.

Zhang, Z-Y., Zhang, X-K., Jhao, J-S., Zhang, X-P., Liang, W-J., 2015. Tillage and rotation effects on community composition and metabolic footprints of soil nematodes in a black soil. *European Journal of Soil Biology*. 66(1), 40-48. <https://doi.org/10.1016/j.ejsobi.2014.11.006>.

Zuber, S.M., Veum, K.S., Myers, R.L., Kitchen N.R., Anderson, S.H., 2020. Role of inherent soil characteristics in assessing soil health across Missouri. *Agricultural & Environmental Letters*. 5(1), 1-5. <https://doi.org/10.1002/ael2.20021>.

SUPPLEMENTARY MATERIAL

Annexure A: Summary of Herbicide Applications for Maize and Soy under the implementation of Conv farming systems for all three cropping seasons and ecotopes. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY).

Cropping season	Crop	Application time	NY	QTY	NV	QTY	HO	QTY
Cropping season 1 (2020 – 2021)	Maize	Preemergence/ burn down	None		None		- glyphosate granules - ammonium sulphate - 2-4D	2000.00 g 800.00 g 1.00 L
		At planting	None		None		None	
		Follow-up 1	None		- Cantron (mesotrion) - autochlor - Lamda	0.25 L 1.00 L 0.07 L	- Cantron (mesotrion) - glyphosate 2 L	0.20 L 2.00 L
		Follow-up 2	None		- Cantron (mesotrion) - autochlor - atrazine - glyphosate	0.25 L 1.00 L 1000.00 g 2.00 L	None	
Cropping season 2 (2021 – 2022)		Preemergence/ burn down	None		None		- Citric acid - Amm SO4 - 2-4D - Villa51 - Slash710 - Pop-up (Real IPM)	45.00 g 850.00 g 1.00 L 0.15 L 2000.00 g 1.00 L
		At planting	- Intelix - Karate 10CS - Titan Boor	1.00 L 0.04 L 2.00 L	- Paraquat - Frontier - Judo Secure - Amm SO4	2.50 L 1.20 L 0.10 L 1.00 L	None	
		Follow-up 1	- Max 357 - Touchdown - Starter MN - Twister	0.03 L 3.00 L 2.00 L 0.05 L	None		None	
		Follow-up 2	- Twister	0.10 L	None		None	
Cropping season 3 (2022 – 2023)		Preemergence/ burn down	None		- Velocity Dry - Glygran - 2.4 D	1000.00 g 2500.00 g 0.50 L	- Citric acid - Amm SO4 - Cantron	45.00 g 850.00 g 0.220 L

							- Metagan Gold	1.10 L
		At planting	- Armour - Dual Gold - Karate - Paraquat - Twister	20.00 g 700.00 g 0.04 L 3.00 L 0.10 L	- Skoffel - Frontier - Lambda	2.50 L 1.20 L 0.01 L	- Citric acid - Amm SO4 - 2-4D - Villa51 - Slash710	45.00 g 850.00 g 1.30 L 0.15 L 2000.00 g
		Follow-up 1	- Max 357 - Touchdown	0.03 L 2.00 L	- Glygran - Frontier - Judo Sec - Velocity Dry	1500.00 g 1.00 L 0.01 L 1000.00 g	- Citric acid - Amm SO4 - 2-4D - Villa51 - Slash710 - Cantron - Metagan Gold	45.00 g 850.00 g 0.20 L 0.10 L 2000.00 g 0.22 L 0.60 L
Cropping season	Crop	Application time	NY	QTY	Nicol de Vos	QTY	Hendrik Odendaal	QTY
Cropping season 1 (2020 – 2021)	Soy	Preemergence/ burn down	None		None		- glyphosate granules - ammonium sulphate - 2-4D	2000.00 g 800.00 g 1.00 L
		At planting	None		- Lamda (insecticide) - metalachlor	0.07 L 1.5 L	None	
		Follow-up 1	None		- glyphosate - Amm SO4	2.00 L 2.00 L	- Amm SO4 - glyphosate	800.00 g 2000.00 g
		Follow-up 2	None		- glyphosate - Amm SO4	2.00 L 2.00 L	- Amm SO4 - glyphosate	800.00 g 2000.00 g
Cropping season 2 (2021 – 2022)		Preemergence/ burn down	None		- Villa51 (wet) - Glygram - 2-4D - Amm SO4	0.15 L 2500.00 g 0.50 L 1000.00 g	- Citric acid - Amm SO4 - 2-4D - Villa51 - Slash710 - Pop-up (Real IPM)	45.00 g 850.00 g 1.00 L 0.15 L 2000.00 g 1.00 L
		At planting	- Metagan G - Karate 10CS, - Titan Boron - Extreme plus	0.80 L 0.04 L 2.00 L 1.00 L	- Paraquat - Frontier - Judo Secure - Amm SO4	2.50 L 1.20 L 0.10 L 1.00 L	None	

Cropping season 3 (2022 – 2023)			- Paraquat - Twister	2.00 L 0.10 L				
		Follow-up 1	- Max 357 - Touchdown - Starter MN - Twister	0.03 L 2.00 L 2.00 L 0.05 L	None		Citric acid Amm SO4 Villa51 1 Roundup Ppwer Pop-up (Real IPM) Boric acid Natrium Mo Mn SO4	45.0 g 850.00 g 0.10 L 3000.00 g 2.50 L 500.00 g 100.00 g 300.00 g
		Follow-up 2	- Max 357 - Touchdown - Hold - Twister	0.03 L 2.00 L 0.50 L 0.10 L	None		None	
		Preemergence/ burn down	None		- Velocity Dry - Glygran - 2.4 D	1000.00 g 2500.00 g 0.50 L	None	
		At planting	- Armour - Dual Gold - Karate - Paraquat - Twister	20.00 g 700.00 g 0.04 L 3.00 L 0.10 L	- Skoffel - Frontier - Lambda	2.50 L 1.20 L 0.01 L	- Citric acid - Amm SO4 - 2-4D - Glyphosate - Classic - Molybdenum - Biostimulants	45.00 g 850.00 g 1.30 L 3.00 L 35.00 g 75.00 g 1.00 L
		Follow-up 1	- Max 357 - Touchdown	0.03 L 2.00 L	- Glygran - Frontier - Judo Sec - Velocity Dry	1500.00 g 1.00 L 0.01 L 1000.00 g	- Citric acid - Amm SO4 - Glyphosate	45.00 g 850.00 g 3.00 L

* Quantity (QTY) applied at litre (L) or grams (g) per hectare

Annexure B: Summary of Herbicide Applications for Maize and Soy under the implementation of Cons farming systems for all three cropping seasons and ecotopes. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY).

Cropping season	Crop	Application time	NY	QTY	NV	QTY	HO	QTY
Cropping season 1 (2020 – 2021)	Maize	Preemergence/ burn down	None		- glyphosate granules - Amm SO4 - paraquat	3000.00 g 1000.00 g 2.00 L	- glyphosate granules - ammonium sulphate - 2-4D	2000.00 g 800.00 g 1.00 L
		At planting	None		None		None	
		Follow-up 1	None		- Cantron (mesotrion) - autochlor - Lamda	0.25 L 1.00 L 0.07 L	- Cantron (mesotrion) - glyphosate 2 L	0.20 L 2.00 L
		Follow-up 2	None		- Cantron (mesotrion) - autochlor - atrazine - glyphsate	0.25 L 1.00 L 1000.00 g 2.00 L	None	
Cropping season 2 (2021 – 2022)		Preemergence/ burn down	- Max 357 - Touchdown - Twister	0.03 L 2.00 L 0.10 L	- Villa51 - Glygram - 2-4D - Amm SO4	0.150 L 2500.00 g 0.50 L 1000.00 g	- Citric acid - Amm SO4 - 2-4D - Villa51 - Slash710 - Pop-up (Real IPM)	45.00 g 850.00 g 1.00 L 0.15 L 2000.00 g 1.00 L
		At planting	- Intellex - Karate 10CS - Titan Boor	1.00 L 0.04 L 2.00 L	- Paraquat - Frontier - Judo Secure - Amm SO4	2.50 L 1.20 L 0.10 L 1.00 L	None	
		Follow-up 1	- Max 357 - Touchdown - Starter MN - Twister	0.03 L 3.00 L 2.00 L 0.05 L	None		None	
		Follow-up 2	- Twister	0.10 L	None		None	
Cropping season 3 (2022 – 2023)		Preemergence/ burn down	- Max 357 - Touchdown - Twister	0.03 L 2.00 L 0.10 L	- Velocity Dry - Glygran - 2.4 D	1000.00 g 2500.00 g 0.50 L	- Citric acid - Amm SO4 - Cantron - Metaqan Gold	45.00 g 850.00 g 0.220 L 1.10 L

		At planting	- Armour - Dual Gold - Karate - Paraquat - Twister	20.00 g 700.00 g 0.04 L 3.00 L 0.10 L	- Skoffel - Frontier - Lambda	2.50 L 1.20 L 0.01 L	- Citric acid - Amm SO4 - 2-4D - Villa51 - Slash710	45.00 g 850.00 g 1.30 L 0.15 L 2000.00 g
		Follow-up 1	- Max 357 - Touchdown	0.03 L 2.00 L	- Glygran - Frontier - Judo Sec - Velocity Dry	1500.00 g 1.00 L 0.01 L 1000.00 g	- Citric acid - Amm SO4 - 2-4D - Villa51 - Slash710 - Cantron - Metagan Gold	45.00 g 850.00 g 0.20 L 0.10 L 2000.00 g 0.22 L 0.60 L
Cropping season	Crop	Application time	NY	QTY	Nicol de Vos	QTY	Hendrik Odendaal	QTY
Cropping season 1 (2020 – 2021)	Soy	Preemergence/ burn down	None		- glyphosate granules - Amm SO4 - paraquat	3000.00 g 1000.00 g 2.00 L	- glyphosate granules - ammonium sulphate - 2-4D	2000.00 g 800.00 g 1.00 L
		At planting	None		- Lamda (insecticide) - metalachlor	0.07 L 1.5 L	None	
		Follow-up 1	None		- glyphosate - Amm SO4	2.00 L 2.00 L	- Amm SO4 - glyphosate	800.00 g 2000.00 g
		Follow-up 2	None		- glyphosate - Amm SO4	2.00 L 2.00 L	- Amm SO4 - glyphosate	800.00 g 2000.00 g
Cropping season 2 (2021 – 2022)		Preemergence/ burn down	- Max 357 - Touchdown - Twister	0.03 L 2.00 L 0.10 L	- Villa51 (wet) - Glygram - 2-4D - Amm SO4	0.15 L 2500.00 g 0.50 L 1000.00 g	- Citric acid - Amm SO4 - 2-4D - Villa51 - Slash710 - Pop-up (Real IPM)	45.00 g 850.00 g 1.00 L 0.15 L 2000.00 g 1.00 L
		At planting	- Metagan G - Karate 10CS, - Titan Boron - Extreme plus - Paraquat	0.80 L 0.04 L 2.00 L 1.00 L 2.00 L	- Paraquat - Frontier - Judo Secure - Amm SO4	2.50 L 1.20 L 0.10 L 1.00 L	None	

			- Twister	0.10 L				
		Follow-up 1	- Max 357 - Touchdown - Starter MN - Twister	0.03 L 2.00 L 2.00 L 0.05 L	None		Citric acid Amm SO4 Villa51 1 Roundup Ppwer Pop-up (Real IPM) Boric acid Natrium Mo Mn SO4	45.0 g 850.00 g 0.10 L 3000.00 g 2.50 L 500.00 g 100.00 g 300.00 g
		Follow-up 2	- Max 357 - Touchdown - Hold - Twister	0.03 L 2.00 L 0.50 L 0.10 L	None		None	
		Preemergence/ burn down	- Max 357 - Touchdown - Twister	0.03 L 2.00 L 0.10 L	- Velocity Dry - Glygran - 2.4 D	1000.00 g 2500.00 g 0.50 L	None	
		At planting	- Armour - Dual Gold - Karate - Paraquat - Twister	20.00 g 700.00 g 0.04 L 3.00 L 0.10 L	- Skoffel - Frontier - Lambda	2.50 L 1.20 L 0.01 L	- Citric acid - Amm SO4 - 2-4D - Glyphosate - Classic - Molybdenum - Biostimulants	45.00 g 850.00 g 1.30 L 3.00 L 35.00 g 75.00 g 1.00 L
Cropping season 3 (2022 – 2023)		Follow-up 1	- Max 357 - Touchdown	0.03 L 2.00 L	- Glygran - Frontier - Judo Sec - Velocity Dry	1500.00 g 1.00 L 0.01 L 1000.00 g	- Citric acid - Amm SO4 - Glyphosate	45.00 g 850.00 g 3.00 L

Annexure C: Summary of Herbicide Applications for summer cover crops (SCC) under the implementation of Regen farming systems for all three cropping seasons and ecotopes. The ecotopes include Hendrik Odendaal (HO), Nicol de Vos (NV), and Nant Yzel (NY).

Cropping season	Crop	Application time	NY	QTY	NV	QTY	HO	QTY
Cropping season 1 (2020 – 2021)	SCC	Preemergence/ burn down	- glyphosate - Amm SO4 - paraquat	2000.00 g 1000.00 g 2.00 L	- glyphosate granules - Amm SO4 - paraquat	3000.00 g 1000.00 g 2.00 L	None	
Cropping season 2 (2021 – 2022)			- Max 357 - Touchdown - Twister	0.03 L 2.00 L 0.10 L	- Villa51 (wet) - Glygram - 2-4D - Amm SO4	0.15 L 2500.00 g 0.50 L 1000.00 g	- Citric acid - Amm SO4 - 2-4D - Villa51 - Slash710 - Pop-up (Real IPM)	45.00 g 850.00 g 1.00 L 0.15 L 2000.00 g 1.00 L
Cropping season 3 (2022 – 2023)			- Max 357 - Touchdown - Twister	0.03 L 2.00 L 0.10 L	- Velocity Dry - Glygran 1.5kg - Multi Grain foliar - molasses - uream	1000.00 g 1500.00 g 1.50 L 2.00 L 10000.00 g	- Citric Acid - Amm SO4 - Glyphosate - Classic - Molybdenum	45.00 g 850.00 g 3.00 L 35.00 g 75.00 g

Annexure D: All parameters measured during the Haney Analysis

Physical Parameters	Volumetric Aggregate Stability			
Biological Parameters	Organic Matter	%	Water Extractable Organic	C % of Total SOC
	Soil Respiration	CO ₂ -C	Microbially Active Carbon	%
	H ₂ O Total Organic C	ppm	Soil Organic Carbon	%
Chemical Parameters	Soil pH	1:1 H ₂ O	K	Kg/ha
	H ₂ O Total N	ppm	H ₃ A Calcium	ppm
	H ₂ O Organic N	ppm	H ₃ A Aluminium	ppm
	H ₃ A Nitrate	ppm	H ₃ A Iron	ppm
	H ₃ A Ammonium	ppm	H ₃ A Sulphur	ppm
	H ₃ A Inorganic Nitrogen	ppm	H ₃ A Zin	ppm
	Total N (Organic + Inorganic)	ppm	H ₃ A Manganese	ppm
	Organic N: Inorganic N	Ratio	H ₃ A Coppe	ppm
	Organic N Release	ppm	H ₃ A Magnesium	ppm
	Organic N Reserve	ppm	H ₃ A Sodium	ppm

	Total available N	ppm	P Saturation (Al/Fe)	%
	N	Kg/ha	P Saturation (Ca)	%
	H3A Total Phosphorus	ppm	Calcium: Magnesium	Ratio
	H3A Inorganic Phosphorus	ppm	Calcium: Magnesium/Aluminium	Ratio
	H3A Organic Phosphorus	ppm	Magnesium: Potassium	Ratio
	Total available Phosphorus	ppm	Potassium: Sodium	Ratio
	P kg/ha	Kg/ha	Calcium	Ca %
	P	Organic: Inorganic	Magnesium	Mg %
	Organic P Release	Ppm	Potassium	K %
	Organic P Reserve	ppm	Sodium	Na %
	H3A Potassium	ppm	Soil Health Score	

Annexure E: Correlation coefficients of physical, chemical, and biological soil health parameters analysed against Principal Component (PC) 1 and PC 2 for cropping season 1 (2020/2021), cropping season 2 (2021/2022), and cropping season 3 (2022/2023). Soil health parameters with a correlation coefficient (r) > 0.7 or r < -0.7 were considered most significant and are highlighted in **bold**.

Physical Parameters				
Cropping season 1	Significant Parameters	PC1	Significant Parameters	PC2
	Sand	0,9051	VAS	-0,0441
	VAS	-0,9501	Sand	-0,0557
	Clay	-0,9557	Clay	-0,1323
Cropping season 2	Sand	0,8089	VAS	0,0161
	Clay	-0,7583	Sand	-0,1636
	VAS	-0,8789	Clay	-0,1673
Cropping season 3	Sand	0,8854	VAS	0,1319
	Clay	-0,8879	Clay	0,0031
	VAS	-0,9211	Sand	-0,0352
Chemical Parameters				
Cropping season 1	Significant Parameters	PC1	Significant Parameters	PC2
	H3A Cu	0,9059	H3A Fe	0,7863
	H3A Mn	0,7704	H3A K	0,7350
	H3A Zn	0,7353	Total N	0,6847
	H3A Fe	0,4502	Total available N	0,6743
	H3A Inorg. N	0,3067	H3A Inorg. N	0,6309
	Total available P	0,2316	H3A Na	0,3849

	pH	-0,1474	H3A S	0,1938
	Total N	-0,2917	H2O Organic N	0,1704
	Total available N	-0,3054	Total available P	0,1326
	H3A K	-0,3264	H3A Mn	0,0762
	H3A Ca	-0,6467	H3A Mg	0,0620
	H3A Na	-0,7231	H3A Cu	-0,1908
	H3A S	-0,8559	H3A Ca	-0,2220
	H2O Organic N	-0,8584	H3A Zn	-0,2288
	H3A Mg	-0,8802	pH	-0,7474
Cropping season 2	H3A Mn	0,9056	H3A Na	0,2407
	H3A Cu	0,7801	H3A Fe	0,1616
	H3A Fe	0,7760	H3A S	0,0376
	H3A Zn	0,6848	H3A Mn	-0,0402
	Total available P	0,6264	H2O Organic N	-0,1087
	H3A Inorg. N	0,1379	H3A K	-0,1611
	pH	0,0695	Total N	-0,2720
	H3A K	-0,1503	Total available P	-0,3074
	H3A Ca	-0,5853	H3A Mg	-0,3448
	Total available N	-0,6055	H3A Inorg. N	-0,3600
	Total N	-0,6646	Total available N	-0,3861
	H2O Organic N	-0,8308	H3A Cu	-0,4670
	H3A Mg	-0,8741	H3A Zn	-0,5267

	H3A Na	-0,8775	H3A Ca	-0,6922
	H3A S	-0,9228	pH	-0,8058
Cropping season 3	H3A Fe	0,8972	H3A Na	0,2235
	H3A Mn	0,8847	H2O Organic N	0,0653
	H3A Cu	0,7632	pH	-0,0623
	H3A Zn	0,6598	H3A Mg	-0,0726
	Total available P	0,3884	H3A Mn	-0,1091
	pH	0,2492	H3A Fe	-0,1194
	H3A K	0,0447	H3A Cu	-0,1304
	H3A Inorg. N	-0,1151	H3A S	-0,1800
	Total available N	-0,3826	H3A Ca	-0,2640
	H3A Ca	-0,6545	H3A K	-0,3792
	Total N	-0,7257	H3A Zn	-0,4747
	H2O Organic N	-0,8728	Total N	-0,5680
	H3A Mg	-0,8747	Total available P	-0,6462
	H3A S	-0,8817	Total available N	-0,8454
	H3A Na	-0,8905	H3A Inorg. N	-0,9394
Biological Parameters				
Cropping season 1	Significant Parameters	PC1	Significant Parameters	PC2
	Organic C:N	-0,3473	MAC	0,0652
	POXC	-0,6867	Soil respiration	-0,0417
	MAC	-0,6886	H2O Total Organic C	-0,1169

	H2O Total Organic C	-0,9369	Organic matter	-0,1653
	Soil respiration	-0,9505	POXC	-0,3468
	Organic matter	-0,9593	Organic C:N	-0,5968
Cropping season 2	MAC	0,0134	Organic C:N	0,6354
	Organic C:N	-0,2423	H2O Total Organic C	0,1969
	POXC	-0,7173	POXC	-0,0108
	Soil respiration	-0,7247	Organic matter	-0,2287
	H2O Total Organic C	-0,9009	Soil respiration	-0,2750
	Organic matter	-0,9395	MAC	-0,5328
Cropping season 3	MAC	0,4308	Organic C:N	0,4506
	Organic C:N	-0,2654	H2O Total Organic C	0,1854
	Soil respiration	-0,4956	Soil respiration	0,1029
	POXC	-0,6506	POXC	0,0004
	H2O Total Organic C	-0,9025	Organic matter	-0,0046
	Organic matter	-0,9372	MAC	-0,0842

Annexure F: Correlation Matrix between main parameters and additional parameters of cropping season 1 (2020/2021). **Organic Matter (OM) (%) (main parameter)**; Soil respiration (CO₂) (C); H₂O Organic C (ppm); **pH (main parameter)** H₃A potassium (K) (ppm); **H₃A iron (Fe) (ppm) (main parameter)**; H₃A sulphur (S) (ppm); H₃A zinc (Zn) (ppm); H₃A manganese (Mn) (ppm); **H₃A copper (Cu) (ppm) (main parameter)**; **H₃A magnesium (Mg) (ppm) (main parameter)**; H₃A sodium (Na) (ppm)

Parameter corelation matrix for cropping season 1 (2020/2021)											
	OM	CO ₂ - C	Organic C	pH	K	Fe	S	Zn	Mn	Cu	Mg
OM											
CO ₂ - C	0,95										
Organic C	0,93	0,91									
pH	0,70	0,83	0,33								
K	0,43	0,32	0,88	-0,31							
Fe	0,28	0,20	-0,37	0,64	0,58						
S	0,80	0,84	0,28	-0,61	0,34	-0,27					
Zn	-0,33	-0,33	0,28	-0,10	-0,40	0,11	-0,60				
Mn	0,21	0,28	-0,19	0,25	-0,01	0,50	-0,65	0,70			
Cu	0,23	0,30	0,23	0,11	-0,37	0,31	-0,84	0,81	0,80		
Mg	-0,20	-0,23	0,67	0,12	0,51	-0,27	0,74	-0,57	-0,50	-0,72	
Na	0,14	0,22	-0,52	0,26	0,56	0,03	0,64	-0,69	-0,36	-0,73	0,69

Annexure G: Correlation Matrix between main parameters and additional parameters of cropping season 2 (2021/2022). Permanganate-Oxidizable Carbon (POXC) (mg/kg); **Organic Matter (OM) (%) (main parameter)**; Soil respiration (CO₂) (C); H₂O Organic C (ppm); **pH (main parameter)** H₂O Organic N (ppm); H₃A iron (Fe) (ppm); **H₃A sulphur (S) (ppm) (main parameter)**; **H₃A manganese (Mn) (ppm) (main parameter)**; H₃A copper (Cu) (ppm); H₃A magnesium (Mg) (ppm); H₃A sodium (Na) (ppm)

Parameter corelation matrix for cropping season 2 (2021/2022)											
	POXC	OM	CO ₂ - C	Organic C	pH	Organic N	Fe	S	Mn	Cu	Mg
POXC											
OM	0,72										
CO ₂ - C	0,53	0,72									
Organic C	0,54	0,78	0,60								
pH	-0,13	0,09	0,15	-0,24							
Organic N	0,48	0,77	0,53	0,89	-0,07						
Fe	-0,50	-0,76	-0,61	-0,61	0,12	-0,49					
S	0,54	0,86	0,63	0,82	0,01	0,68	-0,78				
Mn	-0,62	-0,90	-0,57	-0,75	0,09	-0,69	0,68	-0,81			
Cu	-0,56	-0,62	-0,45	-0,81	0,19	-0,62	0,52	-0,72	0,72		
Mg	0,51	0,95	0,63	0,67	-0,01	0,68	-0,82	0,83	-0,85	-0,48	
Na	0,55	0,74	0,67	0,85		0,68	-0,69	0,88	-0,74	-0,84	0,64

Annexure H: Correlation Matrix between main parameters and additional parameters of cropping season 3 (2022/2023). **Organic Matter (OM) (%) (main parameter)**; H2O Organic C (ppm); H2O Organic N (ppm); **Inorganic N (ppm)**; Total N (ppm); Available N (ppm); **H3A iron (Fe) (ppm) (main parameter)**; H3A sulphur (S) (ppm); H3A manganese (Mn) (ppm); H3A copper (Cu) (ppm); H3A magnesium (Mg) (ppm); **H3A sodium (Na) (ppm) (main parameter)**

Parameter corelation matrix for cropping season 3 (2022/2023)											
	OM	Organic C	Organic N	Organic C	Total N	Available N	Fe	S	Mn	Cu	Mg
OM											
Organic C	0,79										
Organic N	0,75	0,96									
Inorganic N	0,08	-0,07	0,01								
Total N	0,59	0,65	0,74	0,64							
Available N	0,33	0,21	0,30	0,88	0,80						
Fe	-0,81	-0,80	-0,79	0,06	-0,59	-0,22					
S	0,84	0,75	0,75	0,22	0,76	0,46	-0,78				
Mn	-0,89	-0,72	-0,67	-0,03	-0,48	-0,22	0,77	-0,69			

Cu	-0,63	-0,77	-0,74	-0,03	-0,54	-0,26	0,69	-0,52	0,66		
Mg	0,93	0,67	0,61	0,11	0,53	0,30	-0,80	0,82	-0,85	-0,45	
Na	0,76	0,4292	0,87	-0,4544	0,25	-0,05	0,86	-0,15	0,60	0,14	-0,48