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ORIGINAL PAPER

Time Matters: A Short-Term Longitudinal Analysis of Conservation Agriculture and Its Impact on Soil Health

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Abstract

Conservation agriculture is seen as a potential solution to modern farming challenges. This paper elucidates its immediate impact by investigating the short-term dynamics of soil health, including the biological constituents, following the implementation of conservation agriculture. We hypothesised that implementing conservation agriculture would lead to changes in soil health. However, these changes will vary across the physical, chemical, and biological properties of the soil, given that each of these components is likely differently influenced by conservation agriculture. The study site was a multi-year trial in Ottosdal (South Africa) with different crop production systems, including maize monoculture, conservation agriculture (rotation of maize, sunflower, and cover crops), and an uncultivated grassland, which served as a natural reference system. Appropriate statistical methods were used to analyse soil health parameters and their interactions from samples collected during the three consecutive summer growing seasons. The study revealed significant soil health dynamics between the cultivated and uncultivated systems. Total available P, organic matter content, and microbial biomass were key indicators of soil health over the 3 years. Crop sequence influenced these dynamics, while a shift from abiotic to biotic factors was observed as primary system differentiators. Notably, crop rotation and soil structure significantly influenced soil microbial communities. These findings provide valuable insights into the interactions between soils and biota and the resulting effects on soil health dynamics. However, further research is required to fully elucidate the mechanisms involved and optimise sustainable farming practices for diverse environmental contexts.

Keywords Sustainable agriculture · Microbial community structure · Nematode-based indices · Ecosystem functioning

1 Introduction

Soil health is fundamental to sustainable food production by promoting both productivity and environmental health (Bünemann et al. 2018; Lehmann et al. 2020). With the escalating global population and increasingly evident consequences of climate change, the need to enhance soil health is becoming more pressing. Doing so will strengthen the resilience of crop production systems against climatic phenomena and help maintain economic viability in the agricultural sector (Piñeiro et al. 2020). Conservation agriculture (CA) and regenerative agriculture (RA) are emerging as promising approaches to address this need by integrating a system of principles and practices aimed at restoring soil health and reversing the destructive consequences of conventional tillage-based agriculture (McLennon et al. 2021; Smith et al. 2021). Conservation agriculture principles include minimum soil disturbance, increasing crop diversity (including cash crop rotation with cover crops), and building soil armour (permanent soil organic cover), while RA adds the emphasis on maintaining living roots and incorporating livestock grazing (Khangura et al. 2023). Since there was no livestock integration in this trial, CA is the most appropriate term relevant to this work.

Monitoring soil health in agricultural systems enables farmers to evaluate a system's advancement and guide its ongoing management (Haney et al. 2018; Moebius-Clune et al. 2017). This is generally achieved by assessing selected parameters representing the physical, chemical, and biological components of soil health (Moebius-Clune et al. 2017). However, due to the cost and expertise

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required, most soil health assessment frameworks place only limited focus on the biological component (Bünemann et al. 2018; Lehmann et al. 2020). This, even though soil biology, which includes microorganisms, nematodes, protozoa, and microarthropods, plays a critical role in delivering ecosystem functions such as nutrient cycling, carbon (C) regulation, water regulation, and pest and disease management (Creamer et al. 2022). Furthermore, the different components of soil health and their indicators present varying sensitivity and responses to soil and crop management practices (Lehmann et al. 2020; Van Es and Karlen 2019). For example, Van Es and Karlen (2019) evaluated 15 soil health indicators from three long-term trials in North Carolina (USA) and showed that biological indicators were more sensitive to differentiate between long-term management practices. For this reason, our approach sought to augment the standard soil health measurements with a more comprehensive assessment of bioindicators.

This endeavour is in line with previous efforts where soil health dynamics were investigated following multiple years after the implementation of CA, typically in long-term research trials (Martin and Sprunger Christine 2022; Swanepoel and Tshuma 2017; Thierfelder et al. 2013). Yet our perspective adds a novel and necessary dimension. We posit that short-term temporal dynamics in soil health (or the lack thereof) can prove important for understanding the initial stages of transitioning to RA. This can provide immediate feedback on the effect of CA implementation, enabling farmers to make informed decisions and adapt the practices as required. This is especially relevant considering that the local environmental context is increasingly recognised as an important factor influencing the success of CA systems (Newton et al. 2020). Furthermore, studying soil health in the short term can reveal early indicators of problems that might not be visible until much later (e.g. nutrient deficiencies or pest infestations).

Considering this knowledge gap, our interest was in evaluating whether short-term changes in soil health can be evidenced following the implementation of CA. We hypothesised that the implementation of CA would have differential impacts on soil health, manifesting as variations in its physical, chemical, and biological properties. This is based on the understanding that each of these components might be uniquely and intricately affected by CA. To investigate this hypothesis, we monitored soil health dynamics including additional bioindicators of microbial and nematode functional analyses across three consecutive summer growing seasons following the implementation of a CA trial in the North-West Province of South Africa.

2 Material and Methods

2.1 Site Description

The research trial (26°49′19.10″S, 26°0′50.50″E) was located near Ottosdal, a small town in the central region of the North-West Province (South Africa). Situated at an elevation of 1510 m, the area experiences a semi-arid climate with a mean annual evaporation rate ranging from 2400 to 2600 mm. The region receives an average annual rainfall of 400 to 600 mm, primarily during the hot summer months from October to March. The farm features a loamy sand soil composition, which typically comprises 80% sand, 11% silt, and 9% clay, with an organic matter content of no more than 0.9%.

2.2 Trial Design

The research trial is part of a Conservation Agriculture Farmer Innovation Program (CA FIP), initiated by the Maize Trust in 2013. This program's aim is to research and develop appropriate CA systems in the summer grain cropping area of South Africa. A fundamental aspect of this is on-farm experimentation, which evaluates the performance of practices and systems under realistic farming conditions. Consequently, the trial designs are shaped considering the farmer's objectives, such as promoting soil health, along with the logistical and environmental context of the farm (Smith et al. 2021).

For the 7 years preceding the trial, maize was cultivated using no-tillage methods. The only exception was the most recent year (summer of 2017/18) during which sunflower was planted under no-tillage. The trial continued for four consecutive years, with the first year (2018/19) planted in January 2019 (supplementary Table a). The three remaining years were planted in November 2019, December 2020, and January 2022. Sufficient rainfall prior to planting is critical for proper seed germination and establishment. Consequently, different planting dates resulted from varying rainfall patterns and wet conditions at the beginning of the summer periods.

The trial consisted of two main systems, namely (a) maize monoculture and (b) CA (rotation of maize, sunflower, and cover crops). These systems were part of a randomised block design (supplementary Table a), and the soil for both systems was managed under no-tillage principles where no soil disturbance took place other than that caused by the no-tillage planter. Information on additional management practices (i.e. cultivar use, planting design, and fertilisation) is provided in Table 1. Due to the nature of FIP trials, fertilisation varied between seasons based on the experience of the farmer and recommendations of agronomists. No nematicides or fungicides

Table 1 Management practices per year associated with systems of maize monoculture, conservation agriculture, and uncultivated grassland. The top dress was applied 4 weeks after planting. Abbreviations: nitrogen (N), phosphorus (P), and potassium (K)

System		2018/2019	2019/2020	2020/2021	2021/2022
Monoculture: maize (*Zea mays*)	Cultivar	DKC 78-79BR	DKC 7276	PAN 5R-785 BR	DKC 86-65BR
	Plant density	50 cm rows 40 000 plants per ha^{1}	50 cm rows 40 000 plants per ha^{1}	50 cm rows 40 000 plants per ha^{1}	50 cm rows 40 000 plants per ha^{1}
	Fertilisation (kg ha^{-1})	At planting: 31 N + 18 P Top dress: 51 N	At planting: 18 P + 31 N Top dress: 51 N	At planting: 37 N + 18 P + 13 K Top dress: 56 N	At planting: 37 N + 20 P + 15 K Top dress: 56 N
Conservation agriculture: maize	Cultivar	DKC 78-79BR	DKC 7276	PAN 5R-785 BR	DKC 86-65BR
	Plant density	50 cm rows 40 000 plants per ha^{1}	50 cm rows 40 000 plants per ha^{1}	50 cm rows 40 000 plants per ha^{1}	50 cm rows 40 000 plants per ha^{1}
	Fertilisation (kg ha^{-1})	At planting: 31 N + 18 P Top dress: 51 N	At planting: 31 N + 18 P Top dress: 51 N	At planting: 37 N + 18 P + 13 K Top dress: 56 N	At planting: 37 N + 20 P + 15 K Top dress: 56 N
Conservation agriculture: sunflower (*Helianthus annuus*)	Cultivar	Agsun 8251	PAN 7102 CLP	Agsun 5106	PAN 7102 CLP
	Plant density	90 cm rows 40 000 plants per ha^{1}	90 cm rows 40 000 plants per ha^{1}	90 cm rows 40 000 plants per ha^{1}	90 cm rows 40 000 plants per ha^{1}
	Fertilisation (kg ha^{-1})	At planting: 31 N + 18 P Top dress: 51 N	At planting: 31 N + 18 P Top dress: 20 N	At planting: 31 N + 16 P Top dress: 39 N	At planting: 31 N + 16 P + 10 K Top dress: 39 N
Conservation agriculture: cover crop	Cultivar	Agri-Life 12 (Agricol)*	Agri-Life 12 (Agricol)*	Agri-Life 12 (Agricol)*	Mixture*
	Plant density	30 cm rows 25 kg seed per ha^{1}	30 cm rows 25 kg seed per ha^{1}	30 cm rows 25 kg seed per ha^{1}	30 cm rows 25 kg seed per ha^{1}
	Fertilisation (kg ha^{-1})	At planting: 24 N + 13 P Top dress: Zero	At planting: 24 N + 13 P Top dress: Zero	At planting: 35 N + 13 P Top dress: Zero	At planting: 35 N + 13 P Top dress: Zero
Uncultivated	Grassland	N/A	N/A	N/A	N/A

*Agri-Life 12: 44% cowpeas (*Vigna unguiculata*), 20% sunhemp (*Crotalaria juncea*), 12% pearl millet (*Pennisetum glaucum*), 8% forage sorghum (*Sorghum bicolor*), 8% Japanese millet (*Echinochloa esculenta*), 4% Niger (*Guizotia abyssinica*), and 4% dolichos (*Lablab purpureus*)

*Mixture: 37% forage sorghum, 37% cowpeas, 11% dolichos, 7% sunhemp, 4% pearl millet, and 4% radishes (*Raphanus sativus*)

were applied. Herbicides (including glyphosate, mesotrione, s-metolachlor and triazine) were used to control weeds, while lambda-cyhalothrin was used at planting for the control of cutworms in maize and sunflower. An uncultivated grassland, located directly adjacent to the trial area, was included as a reference system. This pristine grassland, devoid of any soil and crop production management history, served as a natural reference system. Three replicates were integrated per system. However, a replicate per block for each of the crops in rotation (CA system) was included, facilitating the evaluation of the sequential effects of crop rotation over time.

2.3 Sampling

The first planting season (2018/19) was not sampled, as this would have reflected the effect of only part of a season. Sampling therefore commenced during the second planting season (2019/2020), approximately 100 days after planting. The first, second, and third sampling intervals occurred in February 2020, March 2021, and April 2022, respectively.

A composite soil sample (consisting of 10 sub-samples) was collected per replicate per crop. For the cultivated systems, these included five in-row and five between-row samples, randomly selected within a 5-m radius of a geolocated point recorded during the first sampling interval. For the uncultivated system, 10 randomly located sub-samples were collected per replicate within a 5-m radius of a geolocated point.

For each sub-sample, the top 20 cm of soil was taken with a soil auger, and the contents of the auger were emptied into a 20-L bucket. Upon completion, the bucket content containing all 10 sub-samples was homogenised. Next, 500-ml aliquots of the homogenised soil samples, one for nematode and one for abiotic analyses, were transferred to plastic bags and stored at room temperature in cooler boxes. A 200-ml

aliquot was also collected for phospholipid fatty acid (PLFA) analysis and stored at −20 °C. On the same day of sampling, all the samples were transported to North-West University (Potchefstroom, South Africa) for further processing. Nematode samples were stored for a maximum of 1 week at 6 °C until further processing. For abiotic properties and PLFA analysis, samples were sent to the Soil Health Support Centre (Klapmuts, South Africa).

2.4 Analysis of Abiotic Parameters of Soil Health

Multiple physico-chemical parameters were analysed, namely total available nitrogen (N) and phosphorus (P), C/N ratio, pH, soil structure, and soil texture. Total available N (mg kg^{-1}) was determined following Haney et al. (2018) and quantified using a Lachat 8000 flow injection analyser (H3A extraction of inorganic N) and Teledyne-Tekmar Torch C:N analyser (water extraction of organic N). Similarly, the total available P (mg kg^{-1}) was measured using a Lachat 8000 flow injection analyser (H3A extraction of inorganic PO_4-P) and a Thermo Scientific ICP-OES (H3A extraction of P). The C/N ratio was calculated as the ratio of water-extractable organic C and N (mg kg^{-1}) (Haney et al. 2018). Soil pH was measured in a 1:1 water extract (Patriquin et al. 1993) using a Hanna (HI9813-6) pH metre. Finally, soil structure was measured using the volumetric aggregate stability test (%) of Solvita (2017), while soil texture was determined using Cornell's rapid soil texture analysis (Schindelbeck et al. 2016).

2.5 Analysis of Biotic Parameters of Soil Health

Biotic parameters included nematode-based indices of soil ecosystem status, signature lipid biomarkers of microbial communities, and soil respiration. Furthermore, and in line with Moebius-Clune et al. (2017), organic matter content and active C were also determined as biological endpoints.

Nematodes were extracted from soil using the decanting and sieving followed by sugar flotation methods (Marais et al. 2017). Next, extracted nematodes were fixed in 4% formaldehyde using the hot/cold fixation method (Van Bezooijen 2006). Finally, nematodes were counted and identified using a Peter's 1-ml capacity counting slide (Marais et al. 2017) on a Nikon Eclipse 50i compound microscope. Using the Nematode Indicator Joint Analysis (NINJA) online tool (Sieriebriennikov et al. 2014), selected nematode-based indices of soil ecosystem status were calculated and included the maturity, enrichment, structure, and channel indices (Ferris et al. 2001). The maturity index is a measure of environmental disturbance with values ranging from 1 to 5. Lower and higher values indicate more disturbed and healthier soil ecosystems, respectively (Du Preez et al. 2022). The enrichment index is used to infer food availability and nutrient enrichment, while the structure index is a measure of food web connectance. Both these indices range from 0 to 100 with higher values indicating greater enrichment and structure, respectively. Finally, the channel index indicates the predominant decomposition pathway of organic matter with lower (<50) and higher (>50) values indicating bacterial and fungal dominated decomposition, respectively (Du Preez et al. 2022).

The method of Buyer and Sasser (2012) was used for PLFA analysis. Briefly, lipid classes were separated by solid phase extraction, transesterified to fatty acid methyl esters (FAMEs), and then analysed by capillary gas chromatography with flame ionisation detection. FAMEs were identified using the MIDI PLFAD1 calibration mix and naming table (Buyer and Sasser 2012). To assess microbial community structure, characteristic fatty acids were classified into structural groups. Shifts in microbial community structure can be observed by changes in the proportions of the microbial structural groups. Normal saturated fatty acids (Nsats) are applied as a general microbial biomarker; mid-chain branched saturated fatty acids (MBsats) are indicative of actinomycetes, terminally branched saturated fatty acids (TBsats) of gram-positive bacteria, monounsaturated fatty acids (Monos) of gram-negative bacteria, and polyunsaturated fatty acids (Polys) of fungi (Willers et al. 2015). Biomass was measured in ng g^{-1}.

Soil respiration (CO_2-C mg kg^{-1}), an indication of microbial activity, was measured using rewetted air-dried soil after 24 h of incubation with a MiniCube CO_2 analyser (Haney et al. 2018). Organic matter content (%), in turn, was measured using the loss on ignition method (Donkin 1991), while active C (mg kg^{-1}), a measure of the fraction of C readily available as a food source to soil organisms, was assessed following the permanganate-oxidizable carbon (POXC) method (Schindelbeck et al. 2016).

2.6 Statistical Analysis

The crop sequences (as part of the CA system), maize monoculture system, and uncultivated system were compared through two-way permutational multivariate analyses of variance (Permanova). Data were log-transformed and normalised before rendering a Euclidean distance-based matrix. This method is appropriate when working with non-normally distributed data (Shaphiro-Wilks, 5% significance) and small sample sizes as in this study (Anderson et al. 2008). Abiotic (i.e. total available P, total available N, C to N ratio, pH, soil structure, and silt, sand, and clay contents) and biotic (i.e. maturity, enrichment, structure, and channel indices, PLFAs: total fungi, microbial, bacteria (gram +/−) and Actinomycetes biomasses, soil respiration, organic matter, and active C content) variables were evaluated from each system. The factor "year" (three levels: year 1, year 2, and year 3; fixed) and the factor "crop sequence" (five levels: monoculture

maize (maize mono), sunflower followed by cover crop (sunflower-cover), cover crop followed by maize (cover crop-maize), maize followed by sunflower (maize-sunflower), and uncultivated grassland; fixed) were tested for interactions in a main design, followed by pairwise comparisons (5% significance).

In the presence of significant interactions, each year was evaluated individually through principal component analysis (PCA), and their variables correlated with principal components 1 and 2, to reveal the greater contributors to the differences among the systems. Next, only the main variables ($r > 0.70$, $r < -0.70$; PC1 and PC2) were evaluated individually through pairwise comparisons in search of significant differences. Variables were also related to each other through regression analyses. Correlations regarded as high (i.e. $r > 0.55$, $r < -0.55$) were further investigated for significant levels ($p < 0.05$) to provide insights into causation effects.

3 Results

3.1 Temporal Patterns and Variable Differentiation Between Systems

Significant differences (Table 2) were observed between crop sequences and the uncultivated system within the three study years when considering all the measured variables. Furthermore, a trend emerged indicating differentiation among crop sequences over time, evidenced by an increase in the dispersion of the data (Fig. 1). Generally, the main variables driving this differentiation were not the same in all three years, except for total available P (mg kg^{-1}), organic matter content (%), and abundance of PLFA groups (indicative of microbial community structure) (Fig. 1; supplementary Tables b and c). Total available P was always significantly higher in the cultivated crops, while the organic matter content and microbial biomass presented the opposite pattern (Figs. 2, 3, and 4). Most of the main variables were significantly correlated with each other.

3.2 Year-by-Year Analysis of Main Abiotic and Biotic Variables

In year 1 (Fig. 1 a and b; supplementary Table c), total available P and N (mg kg^{-1}), soil structure (%), silt (%) (PC1), and sand (%) (PC2) were the main variables contributing to the explained variance when only considering the abiotic variables. In turn, PLFAs (total bacteria biomass (ng g^{-1}) as the strongest variable), organic matter (%) (PC1), and the enrichment index (PC2) were the main biotic variables. Sand and clay content were significantly higher in the maize-sunflower sequence compared to the uncultivated system, while organic matter was significantly higher in the uncultivated system compared to all the other systems (Fig. 2). In year 2 (Fig. 1 d and e; supplementary Table c), again, total available P and N were the main abiotic variables, followed by silt and pH (PC1), clay (%), and sand (PC2). The main biotic variables were the PLFAs (total bacteria as the strongest variable), organic matter content, and soil respiration (CO_2-C mg kg^{-1}) (PC1), followed by the structure and maturity indices (PC2). The maturity index was significantly higher in the cover crop-maize sequence compared to the sunflower-cover crop sequence (Fig. 3). In year 3 (Fig. 1

Table 2 Multivariate pairwise comparisons between crop sequences within each year

	t	*p*
Year 1		
Sunflower-cover crop vs maize mono	0.8032	0.5925
Sunflower-cover crop vs cover crop-maize	1.1050	0.3281
Sunflower-cover crop vs maize-sunflower	0.8550	0.5667
Sunflower-cover crop vs uncultivated	3.4178	**0.0057**
Maize mono vs cover crop-maize	1.1355	0.3113
Maize mono vs maize-sunflower	0.7357	0.6555
Maize mono vs uncultivated	3.1739	**0.0067**
Cover crop-maize vs maize-sunflower	1.1285	0.3207
Cover crop-maize vs uncultivated	2.6683	**0.0120**
Maize-sunflower vs uncultivated	3.7791	**0.0053**
Year 2		
Sunflower-cover crop vs maize mono	0.7742	0.6244
Sunflower-cover crop vs cover crop-maize	1.3758	0.1786
Sunflower-cover crop vs maize-sunflower	0.8546	0.5501
Sunflower-cover crop vs uncultivated	3.2734	**0.0068**
Maize mono vs cover crop-maize	1.0051	0.4287
Maize mono vs maize-sunflower	0.6278	0.7635
Maize mono vs uncultivated	2.3261	**0.0221**
Cover crop-maize vs maize-sunflower	0.9948	0.4174
Cover crop-maize vs uncultivated	2.5864	**0.0142**
Maize-sunflower vs uncultivated	3.1395	**0.0099**
Year 3		
Sunflower-cover crop vs maize mono	1.0587	0.3649
Sunflower-cover crop vs cover crop-maize	1.6969	0.0742
Sunflower-cover crop vs maize-sunflower	0.7352	0.6784
Sunflower-cover crop vs uncultivated	2.3627	**0.0199**
Maize mono vs cover crop-maize	1.2083	0.2645
Maize mono vs maize-sunflower	0.9508	0.4601
Maize mono vs uncultivated	2.4154	**0.0228**
Cover crop-maize vs maize-sunflower	1.4046	0.1576
Cover crop-maize vs uncultivated	2.8346	**0.0088**
Maize-sunflower vs uncultivated	2.1760	**0.0332**

Bold values indicate a significant difference ($p < 0.05$)

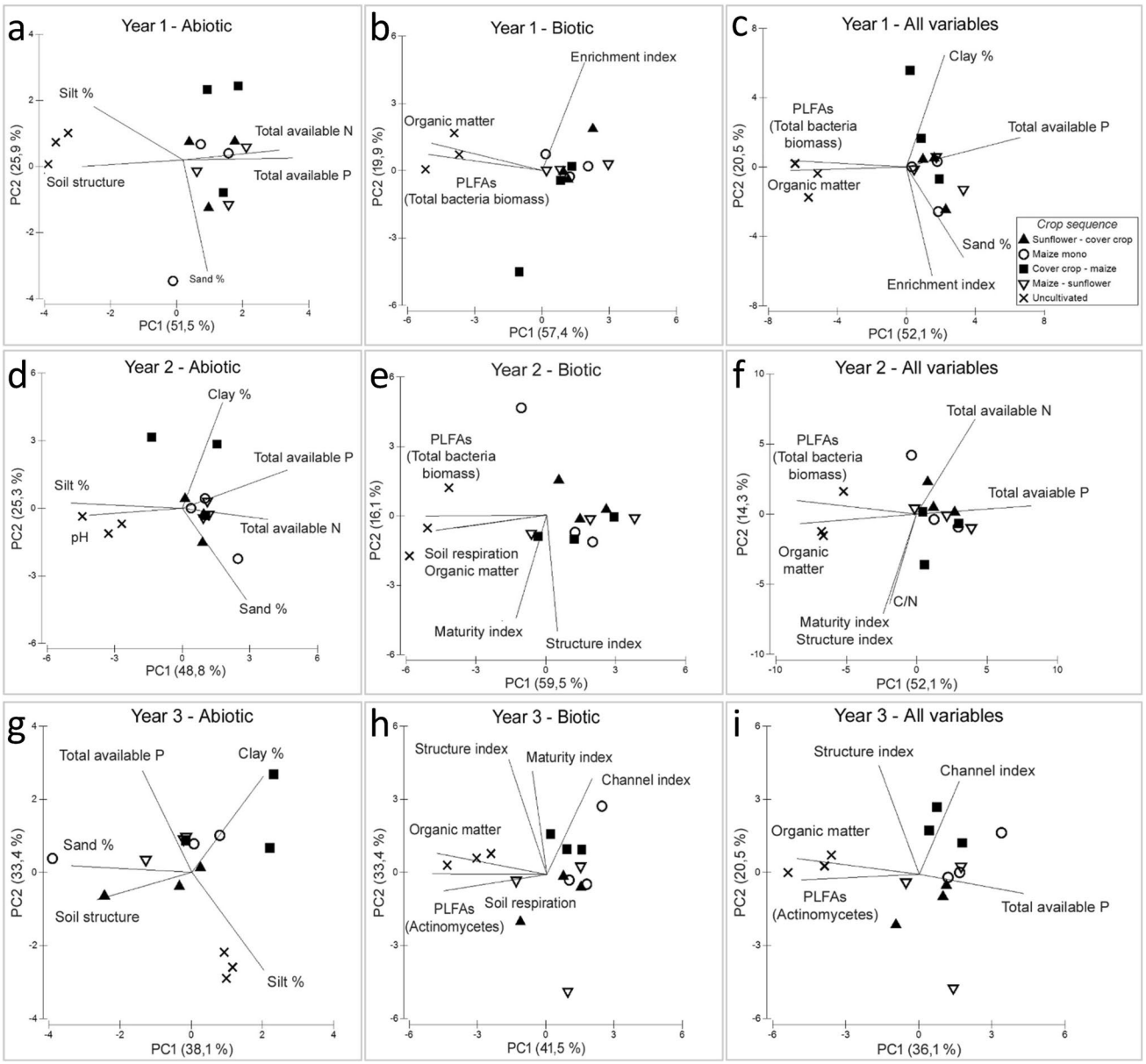

Fig. 1 Principal component analysis ordination of the crop sequences in years 1 (**a**–**c**), 2 (**d**–**f**), and 3 (**g**–**i**), illustrating the abiotic, biotic, and all variables, respectively. Vectors represent the main variables ($r > 0.70$, $r < -0{,}70$; PC1 and PC2) responsible for the distribution of data (see supplementary Table c). The strongest contributing variable of a group (e.g. PLFAs) in which all variables are correlated is shown between parentheses. Abbreviations: nitrogen (N), phosphorus (P), phospholipid fatty acids (PLFAs)

g and h; supplementary Table c), sand and soil structure were the main abiotic variables (PC1), followed by total available P, silt, and clay (PC2). As for the biotic variables, PLFAs (actinomycetes biomass as the strongest variable), organic matter, and soil respiration (PC1) were followed by the structure, maturity, and channel indices (PC2). The structure index was significantly higher in the cover crop-maize sequence and the uncultivated system and lower in the sunflower-cover crop sequence. The channel index, in turn, was significantly lower in the uncultivated system (Fig. 4). It is important to highlight that from year 1 to year 3, there was a shift in the primary contributors to variation, transitioning from non-living (abiotic) to living (biotic) factors.

3.3 Evolving Interplay Between Abiotic and Biotic Variables

Finally, significant correlations (which may imply causation effects) were observed within and between biotic and abiotic variables (supplementary Table d). These

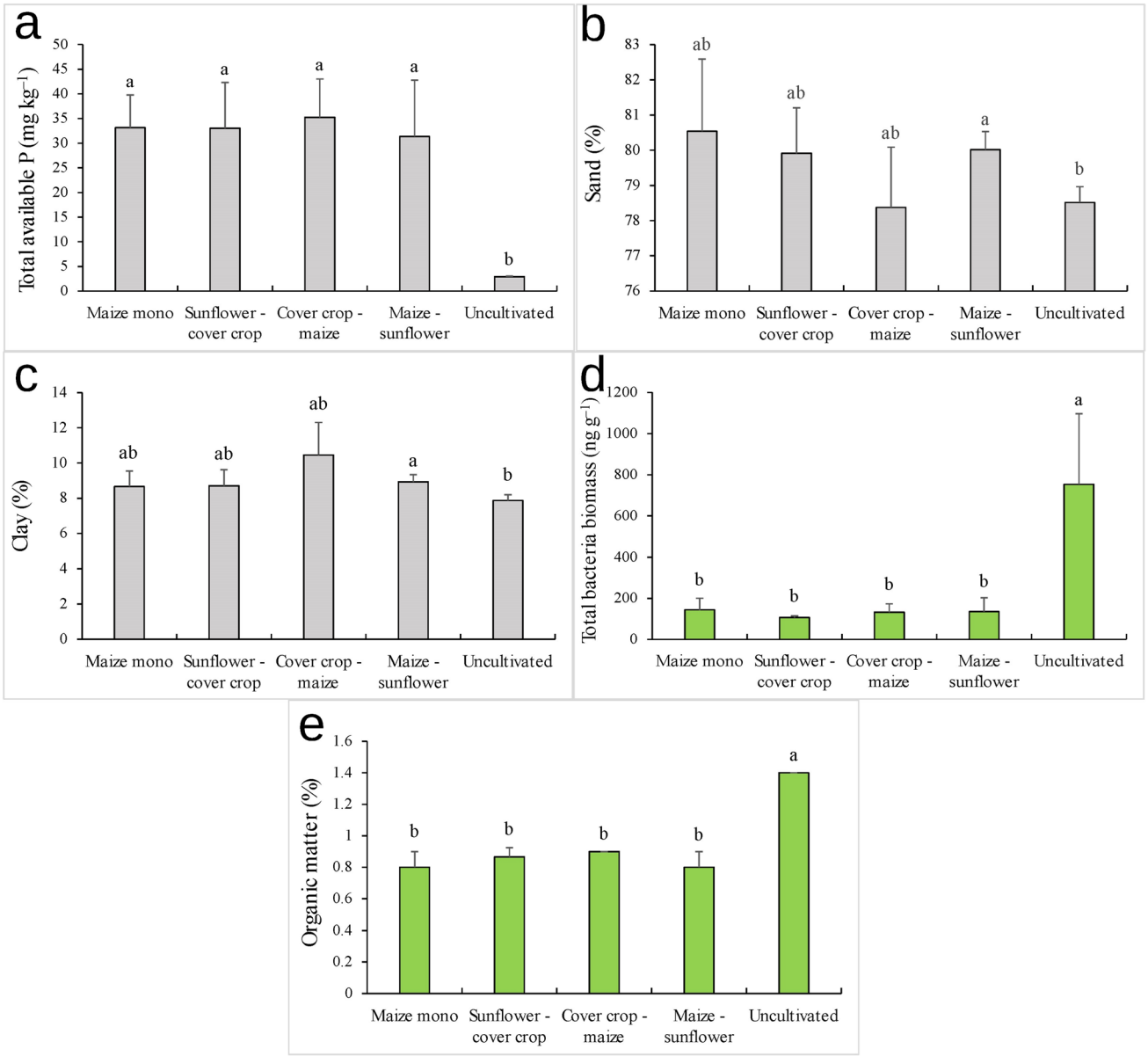

Fig. 2 Comparisons of the main variables (mean values ± sd) (**a**–**e**) that significantly differ among the crop sequences in year 1. Different letters above bars indicate significant differences ($p < 0.05$) while grey and green bars represent abiotic and biotic variables, respectively. Abbreviations: phosphorus (P)

relationships generally changed from year to year. For example, in year 1, total available N was negatively correlated and soil structure positively correlated with PLFAs. Soil structure was also positively correlated with the maturity index, organic matter, soil respiration, and total available N and P. In year 2, pH was positively correlated with PLFAs, while total available N was negatively correlated with the maturity and structure indices. A positive correlation was evidenced between the enrichment index, PLFAs, organic matter, and soil respiration in year 3. Nonetheless, some relationships were more consistent, including the positive correlations evidenced between organic matter, silt, and PLFAs.

4 Discussion

In this study, an uncultivated (natural) grassland was used as an undisturbed reference, which is anchored in the concept that CA is a "nature-based solution." Following this perspective of CA, management practices are implemented to promote ecosystem functioning (Kooijman et al. 2021;

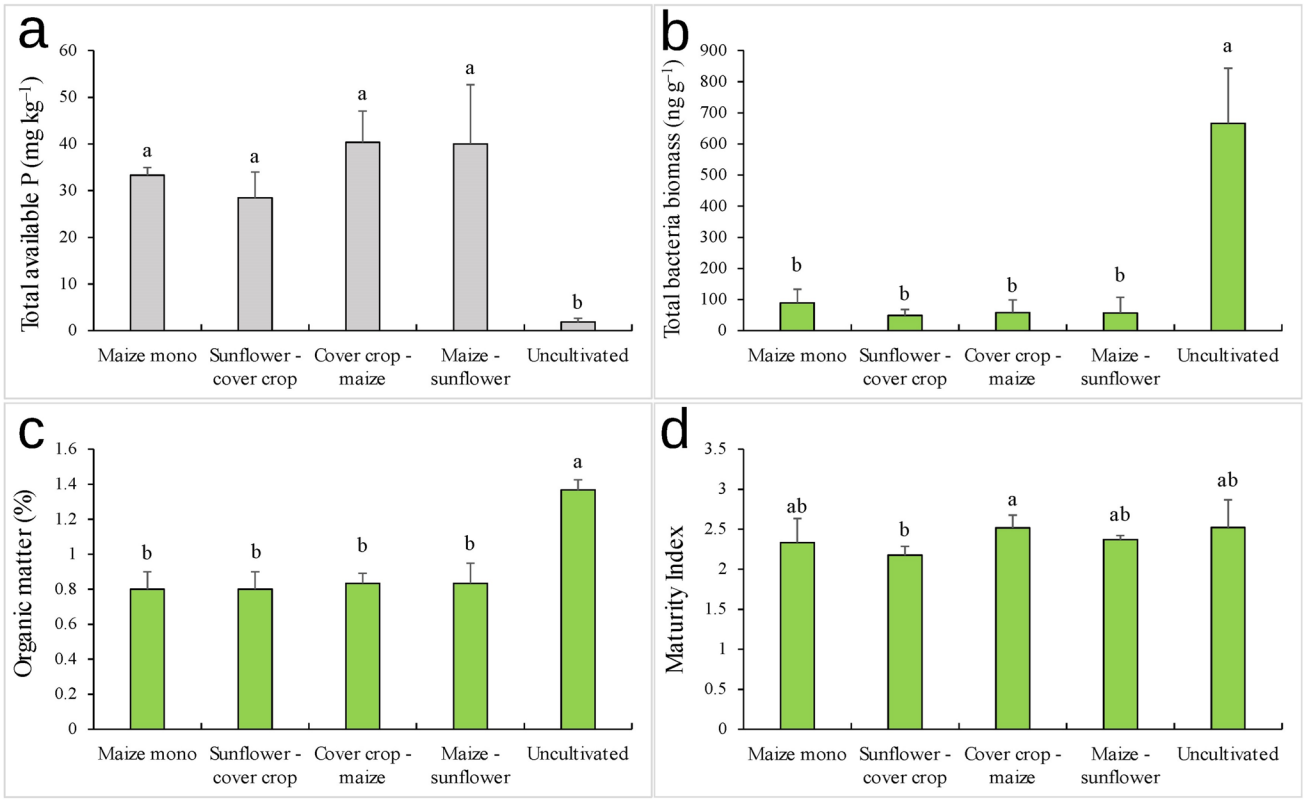

Fig. 3 Comparisons of the main variables (mean values ± sd) (**a**–**d**) that significantly differ among the crop sequences in year 2. Different letters above bars indicate significant differences ($p < 0.05$) while grey and green bars represent abiotic and biotic variables, respectively. Abbreviations: phosphorus (P)

Miralles-Wilhelm and Iseman 2021). Therefore, such a reference system can serve as a baseline, offering insights not only into attainable standards of ecosystem functioning, but also as a model for studying the intricacies of the local undisturbed ecosystem.

Given the contrast between the undisturbed and cultivated systems and the significance of primary indicators such as total available P, organic matter, and PLFAs across the three study years, our findings shed light on the potential of these indicators to inform soil health dynamics in both the agricultural and environmental contexts of our study. The significantly higher levels of total available P (and total available N in years 1 and 2) in the cultivated systems are expected due to the use of inorganic fertilisers (Chen et al. 2014; Shen et al. 2011). Still, nutrient accumulation in the topsoil layers may also be linked to the build-up of plant residues in minimal tillage systems (Duncan et al. 2019). This indicates that the historical practice of minimum tillage at the study site likely had a positive effect on soil fertility (Berner et al. 2008). However, it is also plausible that deeper soil layers had lower nutrient availability (Lynch et al. 2012), which presents a problem in drier periods when roots seek out water and nutrients from deeper soil layers. For this reason, it is critical to protect the upper layers of soil through permanent soil cover with organic material (Thierfelder et al. 2018; Zribi et al. 2015), or as it is commonly known among farmers, building "soil armour." This will help protect the soil from direct exposure to sunlight and reduce evaporation and moisture loss (Zribi et al. 2015).

Conversely, in the uncultivated system, the higher levels of organic matter appear to be a consequence of no management history. Conventional agriculture practices (e.g. tillage and use of agrochemicals) have been found to reduce organic matter content, primarily through physical and chemical disturbances (Man et al. 2021). However, rebuilding soil organic matter in agricultural settings is challenging, and the suggested mechanisms are often contradictory (Averill and Waring 2018; Giller et al. 2021). A key factor to consider is the local environmental context of the study site. Given the soils' high sand and low clay content (see Appendix), the C storage potential of the soils is limited (Giller et al. 2021). Since this study evidenced minimal temporal variation in organic matter, any improvement in organic matter is likely to require multiple growing seasons. Additional practices that can be implemented to help build organic matter content include planting cover crops

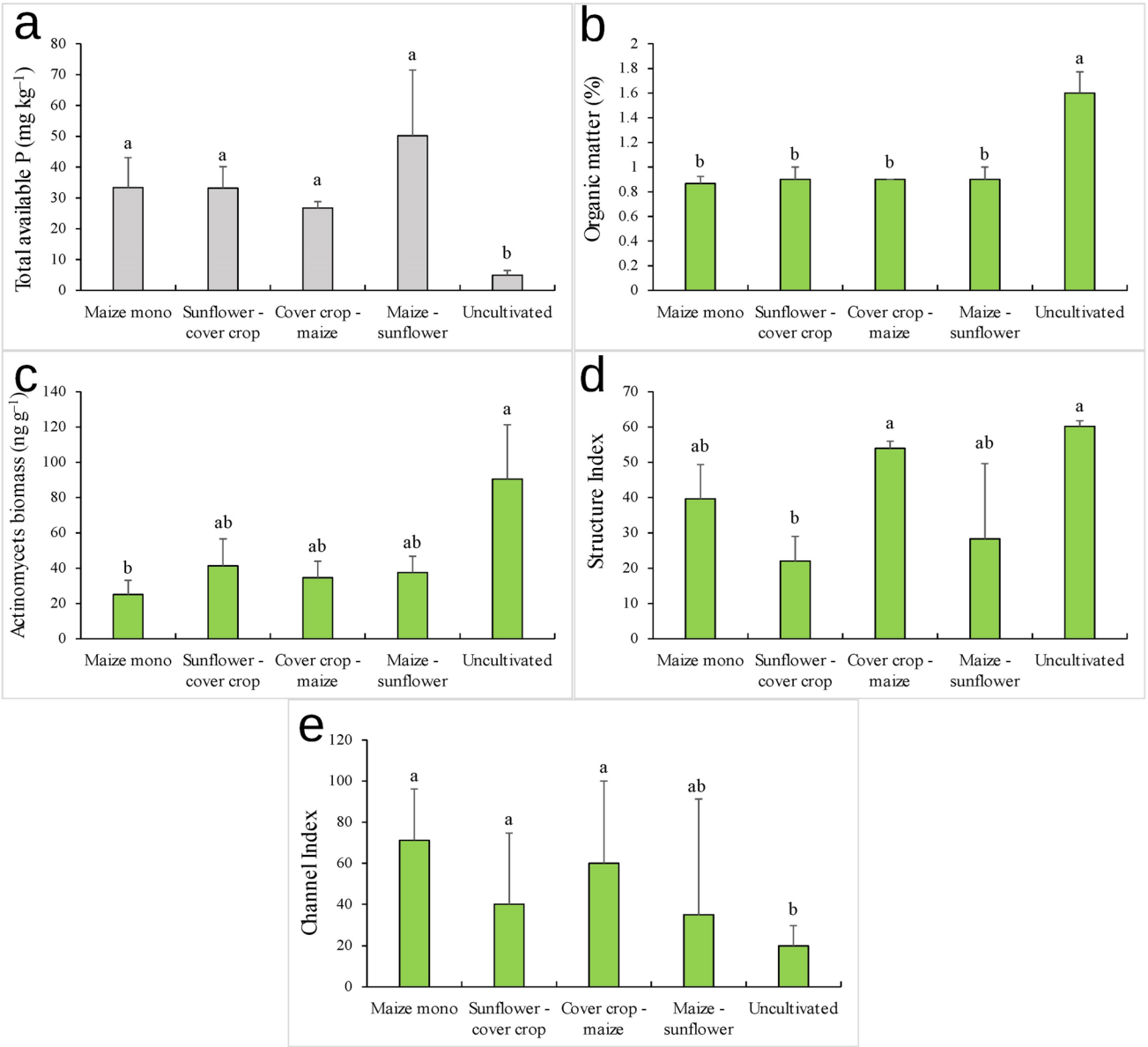

Fig. 4 Comparisons of the main variables (mean values ± sd) (**a**–**e**) that significantly differ among the crop sequences in year 3. Different letters above bars indicate significant differences ($p < 0.05$) while grey and green bars represent abiotic and biotic variables, respectively. Abbreviations: phosphorus (P)

in rotation with cash crops and the integration of livestock through managed grazing (Khangura et al. 2023; Thierfelder et al. 2018). Cover crops add to above and belowground plant biomass, promote root exudation, and reduce soil erosion, thereby promoting organic matter (and C) increases (Adetunji et al. 2020; Strickland et al. 2019). Also, Strickland et al. (2019) found that cover crops and the resulting root exudation in arable fields increased microbial biomass and bioavailable C by 64% and 37%, respectively. Furthermore, the integration of managed grazing aids in utilising some of the cover crops, while the remaining material is trampled and forms a soil cover. The urine and manure from livestock also provide additional nutrients to the soil (Hewins et al. 2018; Sekaran et al. 2021).

A closer examination of the crop sequences revealed that their differentiation was not static but changed from year to year. This finding supports the complexity and interdependency of soil physical, chemical, and biological properties (Bünemann et al. 2018; Creamer et al. 2022). By the third year, the PCAs showed less distinct clustering among systems, while the primary contributors to variation had transitioned from abiotic to biotic factors. While the PLFAs mainly differentiated between the cultivated and uncultivated systems, it was the maturity, structure, and channel indices that accounted

for variation between different crop sequences. These indices presented a strong, positive correlation with the cover crop-maize sequence. Higher values in maturity and structure indicate greater ecosystem health and food web connectance, respectively, while the channel index suggests a shift towards increased fungal decomposition (Du Preez et al. 2022; Ferris et al. 2001). The benefits of these results include greater nutrient cycling due to the flow of resources between trophic levels and the breakdown of recalcitrant organic matter by fungal communities (Creamer et al. 2022). Additionally, it offers potential regulation of pests by omnivore and predator nematodes associated with mature ecosystems (Topalović and Geisen 2023). Therefore, the sequence of planting cover crops followed by maize presents the potential for promoting soil ecosystem health and functionality. Supporting this view, a study by Shackelford et al. (2019) reviewed data from ten meta-analyses and found that planting cover crops led to a 9% increase in organic matter and a 41% increase in microbial biomass. Despite these promising results, the authors highlighted gaps in our understanding of the impact of cover crops (and crop sequence) on soil ecosystem functioning and the interplay between biotic and abiotic properties.

This study's findings on the correlation between PLFAs and soil characteristics, such as organic matter and soil texture, underscore the intricate relationship between soil properties and microbial communities. Under agricultural management, increases in organic C can be linked to increased microbial biomass and fungal abundance (Chen et al. 2020; García-Orenes et al. 2013). A recent study by Chen et al. (2020) utilised a meta-analysis approach of global studies on conservation tillage practices and concluded that soil fungal and bacterial biomass is improved under conservation tillage. Therefore, less intensively managed soils show enhanced organic C content and microbial biomass and activity (García-Orenes et al. 2013). It follows that uncultivated ecosystems without the disturbance of agricultural practices will differ from cultivated systems in terms of microbial community structure, also expecting a more stable community in a less disturbed system. Soil texture has also been shown to correlate with fungal and bacterial biomass due to better protection of organic matter in finer soils (Chen et al. 2020). Furthermore, total bacterial biomass was the strongest contributing variable of the PLFAs in the principal component analysis conducted for years 1 and 2. This can be attributed to the tendency of bacteria to be more abundant in soils that are physically disturbed since tillage breaks the hyphae of fungi (García-Orenes et al. 2013). In addition, bacteria tend to dominate where organic matter is decomposed at a higher rate while fungi dominate in soil with higher contents of recalcitrant organic matter. The latter is indicative of more sustainable agricultural systems (Xu et al. 2020).

The dynamic nature of the studied systems was further evidenced by the shifting relationships within and between abiotic and biotic variables over a temporal scale. In the first year, there was a negative correlation between total available N and total microbial biomass as well as those PLFAs indicative of the different community structure groups (bacteria, fungi, actinomycetes) and organic matter (%). Previous studies have shown that N fertilisation could reduce microbial biomass in many ecosystems (Treseder 2008; Wang et al. 2018). In turn, the positive correlation between soil structure and various soil health indicators points to the role that soil organic matter plays in soil stabilisation, which in turn influenced nutrient cycling (Zhou et al. 2020). Soil pH is another key factor influencing soil ecosystems (An et al. 2021; Kitagami et al. 2020). A significant positive correlation between PLFAs and pH was observed in year 2 with lower pH values measured for all sites compared to year 1 and year 3. According to Joergensen and Wichern (2008), fungal and gram-positive PLFAs may increase as pH decreases. We did not make the same observation in the current study, even though there was a general trend of lower total bacterial biomass observed in all crop rotations during year 2. In year 3, the actinomycete biomass was the strongest contributing variable to PLFAs and was associated with soil respiration and organic matter. Actinomycetes are capable of decomposing more complex polymers in soils and have been associated with increased organic C content in agricultural soils (Xu et al. 2020).

5 Conclusion

Our study presents compelling insights into the interplay between agricultural practices and soil health dynamics, for example, the impact of crop sequence under conservation agriculture. Especially the inclusion of cover crops followed by maize (cover crop-maize sequence) showed potential for promoting soil health. This was indicated by higher ecosystem maturity, food web connectivity, and enhanced fungal decomposition, which lead to enhanced nutrient cycling and potential pest regulation. Furthermore, the interaction between abiotic and biotic factors, for example the correlation between soil texture and microbial community structure, emphasises the importance of environmental conditions and supports the need for context-specific soil health assessments. Therefore, our hypothesis is accepted owing to the evidenced changes in soil health under conservation agriculture also considering the unique and dynamic responses of soil physical, chemical, and biological properties. In summary, our findings underscore the potential of nature-based solutions, such as conservation agriculture, in promoting soil health and ecosystem functioning. The mechanistic insights derived from our study contribute to the broader understanding of soil health and its role in sustainable food production. However, we recognise that further research is needed to elaborate on the involved mechanisms.

Appendix

System	Crop sequence	Replicate	Year	Biological								
				Maturity index	Channel index	Enrichment index	Structure index	Total microbial biomass (ng g-1)	Total bacteria biomass (ng g^{-1})	Actinomycetes biomass (ng g^{-1})	Gram (−) biomass (ng g^{-1})	Total fungi biomass (ng g^{-1})
Conservation agriculture	Sunflower-cover crop	1	1	2.33	10.53	76.00	75.06	474.05	99.44	14.50	29.71	0.00
Conservation agriculture	Sunflower-cover crop	2	1	2.38	100.00	18.75	49.02	535.87	115.85	13.11	41.38	8.82
Conservation agriculture	Sunflower-cover crop	3	1	2.22	100.00	20.00	33.33	666.82	102.29	11.71	47.30	27.28
Maize monoculture	Maize mono	1	1	2.29	100.00	39.13	42.27	500.52	101.99	12.74	37.86	9.96
Maize monoculture	Maize mono	2	1	2.27	69.23	38.24	44.00	839.19	207.38	30.08	74.56	21.24
Maize monoculture	Maize mono	3	1	2.22	100.00	20.00	33.33	633.05	123.13	21.73	37.83	3.43
Conservation agriculture	Cover crop-maize	1	1	2.58	20.00	27.78	65.33	515.75	85.89	10.55	28.63	4.02
Conservation agriculture	Cover crop-maize	2	1	2.30	100.00	20.00	43.86	592.50	156.35	40.20	37.69	5.05
Conservation agriculture	Cover crop-maize	3	1	2.75	60.00	0.00	75.76	765.47	155.84	20.88	51.37	22.86
Conservation agriculture	Maize-sunflower	1	1	2.40	100.00	42.86	50.00	287.21	60.10	5.00	29.49	8.39
Conservation agriculture	Maize-sunflower	2	1	2.38	100.00	33.33	51.02	559.21	155.81	24.81	71.68	43.71
Conservation agriculture	Maize-sunflower	3	1	2.15	100.00	22.58	25.00	657.05	189.34	34.06	65.73	62.79
Uncultivated	Uncultivated	1	1	2.75	60.00	20.26	72.27	1726.62	572.54	99.27	260.68	130.98
Uncultivated	Uncultivated	2	1	2.92	20.00	29.41	80.49	1351.75	537.10	115.66	144.10	57.18
Uncultivated	Uncultivated	3	1	2.58	100.00	11.11	64.04	2801.80	1149.37	204.03	531.56	353.17
Conservation agriculture	Sunflower-cover crop	1	2	2.19	14.29	48.28	48.72	407.13	30.15	3.15	18.27	10.84
Conservation agriculture	Sunflower-cover crop	2	2	2.06	28.57	28.28	22.40	405.18	68.34	17.24	35.72	31.44
Conservation agriculture	Sunflower-cover crop	3	2	2.28	4.76	36.84	51.52	298.25	47.90	10.82	18.40	18.37
Maize monoculture	Maize mono	1	2	2.59	11.11	23.08	68.17	302.30	46.75	9.03	17.26	0.00
Maize monoculture	Maize mono	2	2	2.00	100.00	33.33	0.00	798.52	133.80	25.04	34.92	13.29
Maize monoculture	Maize mono	3	2	2.41	20.00	46.88	62.22	520.75	86.78	0.00	42.93	6.24
Conservation agriculture	Cover crop-maize	1	2	2.35	60.00	21.74	49.12	270.09	28.78	2.54	12.96	0.00
Conservation agriculture	Cover crop-maize	2	2	2.67	100.00	6.98	67.48	438.35	41.75	11.40	12.87	0.00
Conservation agriculture	Cover crop-maize	3	2	2.53	20.00	14.71	63.06	1133.27	104.56	4.29	55.64	28.55
Conservation agriculture	Maize-sunflower	1	2	2.39	71.43	31.11	51.56	256.34	17.89	0.00	8.96	3.68
Conservation agriculture	Maize-sunflower	2	2	2.31	20.00	43.48	55.56	301.94	39.73	5.07	15.53	5.93

System	Crop sequence	Replicate	Year	Biological								
				Maturity index	Channel index	Enrichment index	Structure index	Total microbial biomass (ng g-1)	Total bacteria biomass (ng g^{-1})	Actinomycetes biomass (ng g^{-1})	Gram (−) biomass (ng g^{-1})	Total fungi biomass (ng g^{-1})
Conservation agriculture	Maize-sunflower	3	2	2.41	7.69	32.50	58.94	820.20	113.53	14.08	27.00	3.99
Uncultivated	Uncultivated	1	2	2.17	100.00	8.70	27.59	1164.83	475.71	106.97	116.78	85.51
Uncultivated	Uncultivated	2	2	2.86	20.00	46.88	80.52	2005.52	826.86	200.83	263.50	170.48
Uncultivated	Uncultivated	3	2	2.54	66.67	23.53	60.31	1837.97	696.04	175.93	135.38	22.11
Conservation agriculture	Sunflower-cover crop	1	3	2.14	60.00	24.39	29.55	1066.63	126.05	38.71	0.00	0.00
Conservation agriculture	Sunflower-cover crop	2	3	2.10	0.00	14.29	22.04	1041.09	196.57	57.69	7.85	2.09
Conservation agriculture	Sunflower-cover crop	3	3	2.06	60.00	16.67	14.53	700.69	96.03	27.55	0.00	0.00
Maize monoculture	Maize mono	1	3	2.85	100.00	5.56	72.96	772.09	66.55	22.13	0.00	0.00
Maize monoculture	Maize mono	2	3	2.07	57.89	27.94	20.33	623.47	62.17	19.00	0.00	0.00
Maize monoculture	Maize mono	3	3	2.10	55.56	33.96	25.53	764.06	112.83	34.30	0.00	0.00
Conservation agriculture	Cover crop-maize	1	3	2.25	100.00	8.70	36.36	1005.75	97.35	31.06	0.00	2.90
Conservation agriculture	Cover crop-maize	2	3	2.55	20.00	19.23	63.16	865.22	83.57	27.47	0.00	0.00
Conservation agriculture	Cover crop-maize	3	3	2.54	60.00	13.51	62.30	819.03	159.11	45.27	7.87	0.00
Conservation agriculture	Maize-sunflower	1	3	1.91	0.00	28.57	0.00	1023.73	114.17	31.73	0.00	0.00
Conservation agriculture	Maize-sunflower	2	3	2.35	4.76	44.68	61.19	980.40	180.17	48.25	8.07	0.00
Conservation agriculture	Maize-sunflower	3	3	2.14	100.00	10.81	23.70	875.25	107.37	32.75	0.00	0.00
Uncultivated	Uncultivated	1	3	2.33	30.43	33.82	53.25	1233.26	307.31	87.05	15.85	2.70
Uncultivated	Uncultivated	2	3	2.53	11.11	34.62	68.52	1059.57	207.53	61.46	9.03	2.04
Uncultivated	Uncultivated	3	3	2.30	18.37	52.69	58.78	1777.10	442.62	122.97	22.18	2.49

System	Biological				Chemical				Physical			
	Gram (+) biomass (ng g^{-1})	Soil respiration CO2-C (mg kg^{-1} C)	Organic matter (%)	Active C (mg/kg)	Total available N (mg kg^{-1})	Total available phosphorous (mg kg^{-1})	C/N	pH	Soil structure (volumetric aggregate stability) (%)	Sand (%)	Silt (%)	Clay (%)
Conservation agriculture	69.73	15.20	0.90	92.00	12.60	35.41	14.84	6.00	4.00	81.36	10.95	7.69
Conservation agriculture	74.46	22.90	0.90	96.00	15.00	40.86	11.57	6.10	3.00	79.51	11.51	8.98
Conservation agriculture	55.00	23.70	0.80	68.00	13.20	22.83	24.29	5.70	7.00	78.88	11.67	9.45
Maize monoculture	64.13	16.00	0.70	71.00	8.95	28.12	28.40	5.30	12.00	82.89	9.40	7.70
Maize monoculture	132.81	19.40	0.90	72.00	15.07	30.60	18.00	5.80	13.00	79.12	11.44	9.44
Maize monoculture	85.30	22.50	0.80	68.00	17.02	40.66	17.44	5.90	5.00	79.60	11.58	8.83
Conservation agriculture	57.25	21.00	0.90	70.00	16.83	32.57	26.92	5.30	7.00	80.35	10.85	8.79
Conservation agriculture	118.67	14.30	0.90	66.00	12.80	29.20	20.63	5.80	2.00	77.36	12.50	10.14
Conservation agriculture	104.47	15.70	0.90	69.00	12.10	44.00	14.38	5.90	7.00	77.41	10.16	12.44
Conservation agriculture	30.61	21.20	0.70	76.00	14.20	31.00	22.35	4.90	3.00	80.56	10.82	8.61
Conservation agriculture	84.14	15.80	0.80	67.00	14.60	42.96	10.31	5.70	3.00	79.53	11.08	9.39
Conservation agriculture	123.61	20.80	0.90	72.00	13.97	20.08	19.46	5.70	7.00	79.96	11.26	8.78
Uncultivated	311.85	24.90	1.40	85.00	9.00	2.83	34.00	5.90	15.00	78.10	13.73	8.18
Uncultivated	393.00	49.70	1.40	80.00	8.40	3.00	27.59	5.90	34.00	78.99	13.50	7.50
Uncultivated	617.81	29.40	1.40	69.00	7.50	3.00	20.91	5.90	21.00	78.45	13.64	7.91
Conservation agriculture	11.87	16.92	0.70	84.00	12.53	32.15	14.00	4.84	2.11	81.36	10.95	7.69
Conservation agriculture	32.62	18.36	0.90	109.00	17.94	31.08	10.28	5.15	4.21	79.51	11.51	8.98
Conservation agriculture	29.49	20.55	0.80	92.00	14.40	22.08	12.27	5.30	2.11	78.88	11.67	9.45
Maize monoculture	29.50	18.56	0.70	112.00	14.54	31.49	13.66	4.60	1.57	82.89	9.40	7.70
Maize monoculture	98.88	27.33	0.80	88.00	16.60	33.67	12.57	4.95	2.11	79.12	11.44	9.44
Maize monoculture	43.86	21.37	0.90	85.00	12.80	34.75	13.71	5.06	2.11	79.60	11.58	8.83
Conservation agriculture	15.82	17.12	0.80	93.00	13.24	32.73	14.76	4.78	2.11	80.35	10.85	8.79
Conservation agriculture	28.88	20.00	0.90	95.00	9.66	45.35	63.33	4.86	1.57	77.36	12.50	10.14
Conservation agriculture	48.92	22.95	0.80	68.00	14.10	43.00	12.36	4.95	2.11	77.41	10.16	12.44
Conservation agriculture	8.93	20.27	0.70	104.00	11.87	54.64	16.00	4.75	2.11	80.56	10.82	8.61
Conservation agriculture	24.20	18.63	0.90	107.00	13.35	33.42	12.74	4.60	2.11	79.53	11.08	9.39
Conservation agriculture	86.53	32.19	0.90	86.00	15.40	32.00	11.32	5.13	2.11	79.96	11.26	8.78
Uncultivated	358.92	19.10	1.40	102.00	13.79	2.56	12.80	5.30	12.00	78.10	13.73	8.18
Uncultivated	563.36	36.37	1.40	87.00	9.50	2.00	17.76	5.48	1.57	78.99	13.50	7.50
Uncultivated	560.65	32.33	1.30	114.00	8.30	0.97	27.80	5.59	3.50	78.45	13.64	7.91
Conservation agriculture	126.05	16.58	0.80	72.00	17.56	40.94	13.58	6.12	16.79	81.36	10.95	7.69
Conservation agriculture	188.72	13.15	1.00	58.00	17.93	27.54	13.18	6.26	7.00	79.51	11.51	8.98
Conservation agriculture	96.03	11.78	0.90	68.00	15.24	30.98	13.33	6.11	7.00	78.88	11.67	9.45

System	Biological				Chemical			Physical			
	Gram (+) biomass (ng g^{-1})	Soil respiration CO2-C (mg kg^{-1} C)	Organic matter (%)	Active C (mg/kg)	Total available N (mg kg^{-1})	Total available phosphorous (mg kg^{-1})	C/N	pH	Soil structure (volumetric aggregate stability) (%)	Sand (%)	Silt (%)	Clay (%)
Maize monoculture	66.55	7.26	0.80	74.00	14.28	32.90	14.23	5.98	31.54	82.89	9.40	7.70
Maize monoculture	62.17	10.89	0.90	86.00	10.46	23.83	9.25	6.33	4.21	79.12	11.44	9.44
Maize monoculture	112.83	10.41	0.90	63.00	10.82	43.30	14.32	5.61	4.21	79.60	11.58	8.83
Conservation agriculture	97.35	8.90	0.90	55.00	12.30	28.52	5.38	5.38	7.00	80.35	10.85	8.79
Conservation agriculture	83.57	10.00	0.90	65.00	11.94	27.14	5.77	5.77	7.00	77.36	12.50	10.14
Conservation agriculture	151.24	11.37	0.90	56.00	12.23	24.45	5.74	5.74	4.21	77.41	10.16	12.44
Conservation agriculture	114.17	11.51	0.80	68.00	13.27	30.83	11.72	5.46	15.21	80.56	10.82	8.61
Conservation agriculture	172.11	13.97	1.00	64.00	15.04	73.05	12.55	5.80	4.21	79.53	11.08	9.39
Conservation agriculture	107.37	11.78	0.90	69.00	11.19	46.52	13.53	5.56	4.21	79.96	11.26	8.78
Uncultivated	291.46	22.95	1.50	60.00	14.10	6.76	18.75	5.66	9.11	78.10	13.73	8.18
Uncultivated	198.50	14.45	1.50	66.00	16.39	4.01	7.67	5.42	4.21	78.99	13.50	7.50
Uncultivated	420.45	23.97	1.80	71.00	19.20	4.00	8.66	5.96	9.11	78.45	13.64	7.91

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Declarations

Conflict of Interest The authors declare no competing interests.

References

Adetunji AT, Ncube B, Mulidzi R, Lewu FB (2020) Management impact and benefit of cover crops on soil quality: a review. Soil Tillage Res 204:104717. https://doi.org/10.1016/j.still.2020.104717

An F, Su Y, Niu Z, Liu T, Wang X (2021) Soil nematode community composition, diversity, and soil properties in an age sequence of *Haloxylon ammodendron* plantations in an oasis-desert ecotone of northwestern China. Arid Land Res Manag 35:463–482. https://doi.org/10.1080/15324982.2021.1907484

Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA + for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth

Averill C, Waring B (2018) Nitrogen limitation of decomposition and decay: how can it occur? Glob Change Biol 24:1417–1427. https://doi.org/10.1111/gcb.13980

Berner A, Hildermann I, Fließbach A, Pfiffner L, Niggli U, Mäder P (2008) Crop yield and soil fertility response to reduced tillage under organic management. Soil Tillage Res 101:89–96. https://doi.org/10.1016/j.still.2008.07.012

Bünemann EK, Bongiorno G, Bai Z, Creamer RE, De Deyn G, de Goede R, Fleskens L, Geissen V, Kuyper TW, Mäder P, Pulleman M, Sukkel W, van Groenigen JW, Brussaard L (2018) Soil quality – a critical review. Soil Biol Biochem 120:105–125. https://doi.org/10.1016/j.soilbio.2018.01.030

Buyer JS, Sasser M (2012) High throughput phospholipid fatty acid analysis of soils. Appl Soil Ecol 61:127–130. https://doi.org/10.1016/j.apsoil.2012.06.005

Chen B, Liu E, Tian Q, Yan C, Zhang Y (2014) Soil nitrogen dynamics and crop residues. A review. Agron Sustain Dev 34:429–442. https://doi.org/10.1007/s13593-014-0207-8

Chen H, Dai Z, Veach AM, Zheng J, Xu J, Schadt CW (2020) Global meta-analyses show that conservation tillage practices

promote soil fungal and bacterial biomass. Agric Ecosyst Environ 293:106841. https://doi.org/10.1016/j.agee.2020.106841
Creamer RE, Barel JM, Bongiorno G, Zwetsloot MJ (2022) The life of soils: integrating the who and how of multifunctionality. Soil Biol Biochem 166:108561. https://doi.org/10.1016/j.soilbio.2022.108561
Donkin M (1991) Loss-on-ignition as an estimator of soil organic carbon in A-horizon forestry soils. Commun Soil Sci Plant Anal 22:233–241
Du Preez G, Daneel M, De Goede R, Du Toit MJ, Ferris H, Fourie H, Geisen S, Kakouli-Duarte T, Korthals G, Sánchez-Moreno S, Schmidt JH (2022) Nematode-based indices in soil ecology: application, utility, and future directions. Soil Biol Biochem 169:108640. https://doi.org/10.1016/j.soilbio.2022.108640
Duncan EW, Osmond DL, Shober AL, Starr L, Tomlinson P, Kovar JL, Moorman TB, Peterson HM, Fiorellino NM, Reid K (2019) Phosphorus and soil health management practices. Agric Environ Lett 4:190014. https://doi.org/10.2134/ael2019.04.0014
Ferris H, Bongers T, De Goede RGM (2001) A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Appl Soil Ecol 18:13–29. https://doi.org/10.1016/S0929-1393(01)00152-4
García-Orenes F, Morugán-Coronado A, Zornoza R, Scow K (2013) Changes in soil microbial community structure influenced by agricultural management practices in a Mediterranean agro-ecosystem. PLoS One 8:e80522. https://doi.org/10.1371/journal.pone.0080522
Giller KE, Hijbeek R, Andersson JA, Sumberg J (2021) Regenerative agriculture: an agronomic perspective. Outlook Agric 50:13–25. https://doi.org/10.1177/0030727021998063
Haney RL, Haney EB, Smith DR, Harmel RD, White MJ (2018) The soil health tool — theory and initial broad-scale application. Appl Soil Ecol 125:162–168. https://doi.org/10.1016/j.apsoil.2017.07.035
Hewins DB, Lyseng MP, Schoderbek DF, Alexander M, Willms WD, Carlyle CN, Chang SX, Bork EW (2018) Grazing and climate effects on soil organic carbon concentration and particle-size association in northern grasslands. Sci Rep 8:1336. https://doi.org/10.1038/s41598-018-19785-1
Joergensen RG, Wichern F (2008) Quantitative assessment of the fungal contribution to microbial tissue in soil. Soil Biol Biochem 40:2977–2991. https://doi.org/10.1016/j.soilbio.2008.08.017
Khangura R, Ferris D, Wagg C, Bowyer J (2023) Regenerative agriculture — a literature review on the practices and mechanisms used to improve soil health. Sustainability 15:2338. https://doi.org/10.3390/su15032338
Kitagami Y, Tanikawa T, Matsuda Y (2020) Effects of microhabitats and soil conditions on structuring patterns of nematode communities in Japanese cedar (*Cryptomeria japonica*) plantation forests under temperate climate conditions. Soil Biol Biochem 151:108044. https://doi.org/10.1016/j.soilbio.2020.108044
Kooijman E, McQuaid S, Rhodes M, Collier M, Pilla F (2021) Innovating with nature: from nature-based solutions to nature-based enterprises. Sustainability 13:1263. https://doi.org/10.3390/su13031263
Lehmann J, Bossio DA, Kögel-Knabner I, Rillig MC (2020) The concept and future prospects of soil health. Nat Rev Earth Environ 1:544–553. https://doi.org/10.1038/s43017-020-0080-8
Lynch J, Marschner P, Rengel Z (2012) Chapter 13 - effect of internal and external factors on root growth and development. In: Marschner P (ed) Marschner's Mineral Nutrition of Higher Plants, Third edn. Academic Press, San Diego, pp 331–346
Man M, Wagner-Riddle C, Dunfield KE, Deen B, Simpson MJ (2021) Long-term crop rotation and different tillage practices alter soil organic matter composition and degradation. Soil Tillage Res 209:104960. https://doi.org/10.1016/j.still.2021.104960
Marais M, Swart A, Fourie H, Berry SD, Knoetze R, Malan AP (2017) Techniques and procedures. In: Fourie H, Spaull VW, Jones RK, Daneel MS, De Waele D (eds) Nematology in South Africa: A view from the 21st century. Springer, Cham, pp 73–118
Martin T, Sprunger Christine D (2022) Sensitive measures of soil health reveal carbon stability across a management intensity and plant biodiversity gradient. Front Soil Sci 2. https://doi.org/10.3389/fsoil.2022.917885
McLennon E, Dari B, Jha G, Sihi D, Kankarla V (2021) Regenerative agriculture and integrative permaculture for sustainable and technology driven global food production and security. Agron J 113:4541–4559. https://doi.org/10.1002/agj2.20814
Miralles-Wilhelm F, Iseman T (2021) Nature-based solutions in agriculture: the case and pathway for adoption. Food and Agriculture Organization of the United Nations, Virginia
Moebius-Clune BN, Moebius-Clune DJ, Gugino BK, Idowu OJ, Schindelbeck RR, Ristow AJ, Van Es HM, Thies JE, Shayler HA, McBride MB, Kurtz KSM, Wolfe DW, Abawi GS (2017) Comprehensive assessment of soil health - the Cornell framework. Cornell University, Ithaca
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. Front Sustain Food Syst 4. https://doi.org/10.3389/fsufs.2020.577723
Patriquin DG, Blaikie H, Patriquin MJ, Yang C (1993) On-farm measurements of pH, electrical conductivity and nitrate in soil extracts for monitoring coupling and decoupling of nutrient cycles. Biol Agric Hortic 9:231–272. https://doi.org/10.1080/01448765.1993.9754638
Piñeiro V, Arias J, Dürr J, Elverdin P, Ibáñez AM, Kinengyere A, Opazo CM, Owoo N, Page JR, Prager SD, Torero M (2020) A scoping review on incentives for adoption of sustainable agricultural practices and their outcomes. Nat Sustain 3:809–820. https://doi.org/10.1038/s41893-020-00617-y
Schindelbeck R, Moebius-Clune B, Moebius-Clune D, Kurtz K, Van Es H (2016) Cornell University comprehensive assessment of soil health laboratory standard operating procedures. Cornell University, Ithaca
Sekaran U, Lai L, Ussiri DAN, Kumar S, Clay S (2021) Role of integrated crop-livestock systems in improving agriculture production and addressing food security – a review. J Agricultural Sci Food Res 5:100190. https://doi.org/10.1016/j.jafr.2021.100190
Shackelford GE, Kelsey R, Dicks LV (2019) Effects of cover crops on multiple ecosystem services: ten meta-analyses of data from arable farmland in California and the Mediterranean. Land Use Policy 88:104204. https://doi.org/10.1016/j.landusepol.2019.104204
Shen J, Yuan L, Zhang J, Li H, Bai Z, Chen X, Zhang W, Zhang F (2011) Phosphorus dynamics: from soil to plant. Plant Physiol 156:997–1005. https://doi.org/10.1104/pp.111.175232
Sieriebriennikov B, Ferris H, De Goede RGM (2014) NINJA: an automated calculation system for nematode-based biological monitoring. Eur J Soil Biol 61:90–93. https://doi.org/10.1016/j.ejsobi.2014.02.004
Smith H, Trytsman G, Nel A (2021) On-farm experimentation for scaling-out conservation agriculture using an innovation systems approach in the North-West Province, South Africa. In: Mkomwa S, Kassam A (eds) Conservation Agriculture in Africa: Climate Smart Agricultural Development. CABI, pp 416–430
Solvita (2017) Solvita volumetric aggregate stability test. Woods End Laboratory, Augusta
Strickland MS, Thomason WE, Avera B, Franklin J, Minick K, Yamada S, Badgley BD (2019) Short-term effects of cover crops on soil microbial characteristics and biogeochemical processes across actively managed farms. Agrosyst Geosci Environ 2:180064. https://doi.org/10.2134/age2018.12.0064
Swanepoel PA, Tshuma F (2017) Soil quality effects on regeneration of annual Medicago pastures in the Swartland of South Africa. Afr J Range Forage Sci 34:201–208. https://doi.org/10.2989/10220119.2017.1403462

Thierfelder C, Baudron F, Setimela P, Nyagumbo I, Mupangwa W, Mhlanga B, Lee N, Gérard B (2018) Complementary practices supporting conservation agriculture in southern Africa. A review. Agron Sustain Dev 38:16. https://doi.org/10.1007/s13593-018-0492-8

Thierfelder C, Mwila M, Rusinamhodzi L (2013) Conservation agriculture in eastern and southern provinces of Zambia: long-term effects on soil quality and maize productivity. Soil Tillage Res 126:246–258. https://doi.org/10.1016/j.still.2012.09.002

Topalović O, Geisen S (2023) Nematodes as suppressors and facilitators of plant performance. New Phytol 238:2305–2312. https://doi.org/10.1111/nph.18925

Treseder KK (2008) Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecol Lett 11:1111–1120. https://doi.org/10.1111/j.1461-0248.2008.01230.x

Van Bezooijen J (2006) Methods and techniques for nematology. Wageningen University, Wageningen

Van Es HM, Karlen DL (2019) Reanalysis validates soil health indicator sensitivity and correlation with long-term crop yields. Soil Sci Soc Am J 83:721–732. https://doi.org/10.2136/sssaj2018.09.0338

Wang C, Liu D, Bai E (2018) Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. Soil Biol Biochem 120:126–133. https://doi.org/10.1016/j.soilbio.2018.02.003

Willers C, Jansen van Rensburg PJ, Claassens S (2015) Phospholipid fatty acid profiling of microbial communities–a review of interpretations and recent applications. J Appl Microbiol 119:1207–1218. https://doi.org/10.1111/jam.12902

Xu Z, Zhang T, Wang S, Wang Z (2020) Soil pH and C/N ratio determines spatial variations in soil microbial communities and enzymatic activities of the agricultural ecosystems in Northeast China: Jilin Province case. Appl Soil Ecol 155:103629. https://doi.org/10.1016/j.apsoil.2020.103629

Zhou M, Liu C, Wang J, Meng Q, Yuan Y, Ma X, Liu X, Zhu Y, Ding G, Zhang J, Zeng X, Du W (2020) Soil aggregates stability and storage of soil organic carbon respond to cropping systems on Black Soils of Northeast China. Sci Rep 10:265. https://doi.org/10.1038/s41598-019-57193-1

Zribi W, Aragüés R, Medina E, Faci JM (2015) Efficiency of inorganic and organic mulching materials for soil evaporation control. Soil Tillage Res 148:40–45. https://doi.org/10.1016/j.still.2014.12.003