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Conservation Agriculture Practices Can Improve Earthworm Species Richness and Abundance in the Semi-Arid Climate of Eastern Cape, South Africa

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Abstract: Earthworms play a pivotal role in the regulation of soil health. Studies that explore the effects of conservation agriculture (CA) principles on earthworms under the semi-arid climate of the central Eastern Cape (EC) of South Africa (SA) are limited. Therefore, this study investigated the effects of tillage, crop rotations, and residue management on earthworms’ abundance and species richness. The study design followed a split-split plot with three replicates. The main plot was allocated to tillage treatment, which had conventional tillage (CT) and no-tillage (no-till) as factors. Crop rotation treatment was allocated to a subplot, and had maize (Zea mays)–fallow–maize (MFM), maize–fallow–soybean (Glycine max) (MFS), maize–wheat (Triticum aestivum)–maize (MWM), and maize–wheat–soybean (MWS). Residue management was in the sub-subplot with residue retention and residue removal. The study was carried out over four cropping seasons: summer 2015–2016, winter 2016, spring 2016, and summer 2016–2017. The results showed that the genera Amynthas and Lumbricus, both belonging to the anecic group, and Dendrobaena, belonging to the epigeic group, were present. Earthworm species diversity and density were highest under no-till than under CT. Residue management improved earthworm density regardless of tillage management. Rotations that had fallow periods recorded lower earthworm numbers as compared to continuous cropping systems where wheat was grown in winter. The study concluded that maize–wheat–soybean (MWS) rotation with residue retention results in the highest earthworm abundance and species richness.

Keywords: conservation agriculture; earthworms; diversity; density; biomass; soil organic carbon

1. Introduction

Conservation agriculture (CA) is a climate-smart strategy that improves soil health [1]. This strategy involves three interlinked principles of diversified crop rotations, no or little mechanical disturbance of the soil through tillage and the retention of organic residues on the soil surface [2]. If applied correctly, the aforementioned principles of CA can increase soil organic matter (SOM) and consequently, soil organic carbon (SOC) [3,4]. Soil organic matter, which can be estimated from SOC, is considered a key attribute of soil fertility and productivity because of its influence on the physical, chemical, and biological properties of soil [4]. Earthworms are an important component of soil biota and key in the regulation of organic matter dynamics as well as maintenance of soil structure and fertility. The earthworm’s bioturbation effect facilitates the decomposition of plant litter and other organic substrates and in turn,
increases microbial activity, nutrient availability, and plant uptake [5–7]. The primary food source for earthworms is organic matter in its various stages of decay [8]. This suggests that any management practice that improves SOM such as CA practices could result in greater earthworm abundance and diversity. However, there is little understanding of the effect of CA principles on SOM dynamics and consequently, earthworms’ diversity and numbers.

Research suggests that earthworms play a positive role under CA systems. For example, the addition of earthworms into the soil increases vermicompost production due to increased microbial activity and bioturbation [9]. Vermicompost increases soil’s porosity and water holding capacity by creating vertical and horizontal burrows within the soil, through which oxygen and water can enter, and the emission of carbon dioxide can take place [10]. Increased water holding capacity is particularly key for crop production under semi-arid environments where rainfall is unreliable. Earthworms are also referred to as “bio-engineers”, often reducing the requirement for tillage. Other advantages include control of soil metal concentrations, aeration, pH, and electrical conductivity [11]. In CA systems where residue retention is observed, the burrowing activity by earthworms can promote residue decomposition, humus formation, and nutrient cycling [9]. On the other hand, tilling of soil negatively affects earthworm diversity and density directly through injury caused by cultivation or indirectly due to habitat disruption and reduction in food sources [12].

Various studies have been done in the localities of the Eastern Cape on the effects of CA on the physicochemical properties of soil [13,14]. However, studies that describe the effects of CA principles on earthworms are lacking, especially under the semi-arid environment of the central Eastern Cape of South Africa. Knowledge of the influence of CA practices on earthworms and their relationship with soil organic matter dynamics is imperative for the development of sustainable agroecosystems. Therefore, this study was carried out to investigate the effects of tillage, crop rotations, and residue management on earthworm species richness and abundance in the semi-arid environments of the Alice Jozini ecotope, in central Eastern Cape, South Africa. The study hypothesis was that CA principles such as tillage, crop rotations, and residue management will interact to influence earthworm species richness and abundance in the semi-arid of South Africa.

2. Methods and Materials

2.1. Experimental Site

A field trial was done at the University of Fort Hare (UFH) Research Farm (32°46′15.8″ S and 26°50′52.3″ E). The farm lies at an average altitude of 508 m above sea level and receives an average rainfall of 575 mm annually. The climate in this region is semi-arid, with an annual mean temperature of 18 °C [13]. The soils at the UFH Research Farm are of alluvial origin and can be classified as Haplic Cambisol using the International Union of Soil Sciences (IUSS) Working Group World Reference Base (WRB) [15]. The soil’s texture is 64.2% sand, 16.0% silt, and 19.8% clay [16]. Before the establishment of the trial, the site had been under continuous cultivation of forage lucerne (Medicago sativa) for more than five years.

2.2. Treatments and Design

A split-split plot design was used. The main plot was tillage and had two levels, namely no-till and conventional tillage (CT). Crop rotation was the subplot factor and had four levels, namely maize–fallow–maize (MFM), maize–fallow–soybean (MFS), maize–winter wheat–maize (MWM), and maize–winter wheat–soybean (MWS). Lastly, the sub-subplot factor was residue management at two levels, with residue retention on the soil surface (R+) and with complete residue removal (R–). The resulting treatment combinations were 16, and these were replicated 3 times. The sub-subplot sizes measured 5 × 7 m.
2.3. Agronomic Practices

Land was ploughed, disked, and harrowed before the commencement of the experiment to ensure homogeneity. Thereafter, plots designated to receive CT were tilled before each cropping season, whilst no tilling was done to no-till plots. A short season and prolific maize cultivar (BG 5785BR) was planted in summer. The targeted maize plant population was 250,000 plants ha\(^{-1}\). An early maturing spring wheat cultivar (SST015) was planted in winter, at a seeding rate of 100 kg ha\(^{-1}\). Soybean cultivar (PAN 5409RG) was inoculated with *Rhizobium leguminosarium* and was sown in summer, targeting a population of 250,000 plants ha\(^{-1}\). Only maize planted in summer received chemical fertilizer. At planting, compound fertilizer (6.7% N; 10% P; 13.3% K + 0.5% Zn) was administered to supply 30 kg N, 45 kg P, and 60 kg ha\(^{-1}\) K. At six weeks after planting (WAP), limestone ammonium nitrate (LAN) was applied to supply the remaining 60 kg ha\(^{-1}\) required. The experiment was solely rain-fed.

A summary of crops in rotation since the beginning of the trial is given in Table 1.

Table 1. Summary of the crop rotations done at the University of Fort Hare (UFH) research farm.

| Crop Rotation | Summer 2012–2013 | Winter 2013 | Summer 2013–2014 | Winter 2014 | Summer 2014–2015 | Winter 2015 | Summer 2015–2016 | Winter 2016 | Summer 2016–2017 |
|----------------|------------------|------------|------------------|------------|------------------|------------|------------------|------------|------------------|
| MFM            | Maize            | Fallow     | Maize            | Fallow     | Maize            | Fallow     | Maize            | Fallow     | Maize            |
| MFS            | Maize            | Fallow     | Soybean          | Fallow     | Maize            | Fallow     | Soybean          | Fallow     | Maize            |
| MWM            | Maize            | Wheat      | Maize            | Wheat      | Maize            | Wheat      | Maize            | Wheat      | Maize            |
| MWS            | Maize            | Wheat      | Soybean          | Wheat      | Maize            | Wheat      | Soybean          | Wheat      | Maize            |

Summer season months are October, November, December, January, and February. Winter season months are May, June, July, and August. MFM—maize–fallow–maize; MFS—maize–fallow–soybean; MWM—maize–wheat–maize; MWS—maize–wheat–soybean.

2.4. Data Collection

2.4.1. Earthworm Sampling and Identification

A quadrat, measuring 0.35 × 0.35 m, was randomly placed in each plot. Earthworms present within the quadrat area were made to emerge by applying 4 L of mustard seed (*Brassica alba*) solution, as suggested by Moos et al. [7]. Earthworms that emerged to the surface were hand-picked. Thereafter, soil was excavated to a depth of 0.5 m to capture the inactive and deep surface earthworm dwellers, as suggested by Kamota et al. [17]. Earthworms that were sampled from each plot were counted, after which the earthworms were ethically euthanized using 70% ethanol and stored in formalin for later classification. Earthworms were identified to genera level following procedures described by the Natural Resources Research Institute (NRRI) [18] and Discover Life Organization [19]. Earthworms were then dried at 60 °C for 48 h and weighed to obtain the dry earthworm biomass. The “ash-free-dry-biomass” was calculated as described by NRRI [18]. This involved placing the dry earthworms in a muffle furnace at 500 °C for about 5 h to burn off all the combustible parts of the earthworm. The ash-free dry biomass of the earthworm was then calculated by subtracting the ash contents of the earthworm from the dry weight. To calculate the diversity index (H’) and evenness (E) of the earthworms at each site, the following equations were used (Equations (1) and (2)):

\[
H' = - \sum_{i=1}^{n} p_i \ln p_i \tag{1}
\]

\[
E = \frac{H}{H_{\text{max}}} \tag{2}
\]

where:

- Sum = summation of all pi values;
- \(p_i\) = the number of individual of species I or total number of samples;
- \(N\) = population size of earthworms.
H_{max} = maximum diversity possible H_{max} = \ln (N);
E = species evenness.

Soil water content from 20 cm depth was measured using a Hydro Sense II moisture probe (Campbell Scientific, Inc., Logan, UT, USA) with a total of three measurements across each plot at each earthworm sampling. Earthworm sampling was conducted on 13 March 2016, 27 July 2016, 29 August 2016, and 10 February 2017, representing the summer 2015–2016, winter 2016, spring 2016, and summer 2016–2017 seasons, respectively. The summary of weather elements during the growing period is given in Table 2.

Table 2. Summary of weather elements during the growing period at the UFH research farm.

| Months | Tx     | Tn     | RHx   | RHn   | Rs    | Rain  | ETO   | HU   |
|--------|--------|--------|-------|-------|-------|-------|-------|------|
| Jan 2016 | 30.72  | 16.98  | 88.18 | 36.11 | 28.63 | 0.81  | 5.76  | 12.94 |
| Feb 2016 | 29.38  | 15.73  | 89.91 | 38.83 | 24.14 | 2.94  | 4.73  | 11.69 |
| Mar 2016 | 27.2   | 14.37  | 90.69 | 41.9  | 19.78 | 2.42  | 3.77  | 10.07 |
| Apr 2016 | 26.69  | 10.35  | 91.02 | 32.11 | 16.27 | 1.45  | 2.97  | 7.74  |
| May 2016 | 23.34  | 7.47   | 88.84 | 34.85 | 11.04 | 0.25  | 1.93  | 4.92  |
| Jun 2016 | 22.1   | 5.11   | 88.56 | 28.88 | 8.07  | 0.44  | 1.63  | 2.91  |
| Jul 2016 | 20.22  | 4.78   | 86.08 | 31.64 | 9.15  | 1.42  | 1.78  | 2.15  |
| Aug 2016 | 24.23  | 6.66   | 86.71 | 25.25 | 12.95 | 0.35  | 2.94  | 4.95  |
| Sep 2016 | 22.93  | 8.36   | 89.93 | 34.49 | 15.94 | 1.09  | 2.85  | 4.67  |
| Oct 2016 | 24.53  | 9.69   | 87.57 | 31.08 | 21.06 | 0.61  | 3.82  | 6.6   |
| Nov 2016 | 25.18  | 12.61  | 89.73 | 42.26 | 23.06 | 1.35  | 4.29  | 8.05  |
| Dec 2016 | 30.75  | 14.57  | 85.52 | 30.33 | 25.26 | 0.45  | 5.09  | 11.92 |
| Jan 2017 | 27.95  | 15.04  | 89.97 | 42.03 | 20.64 | 2.9   | 4.09  | 10.7  |
| Feb 2017 | 28.36  | 16.42  | 90.61 | 41.32 | 21.04 | 3.41  | 4.56  | 11.21 |

Tx—Maximum Daily Temperature [°C]; Tn—Minimum Daily Temperature [°C]; RHx—Maximum Daily Relative Humidity [%]; RHn—Minimum Daily Relative Humidity [%]; Rs—Radiation (MJ/m²); ETO—evapotranspiration [mm]; HU—Daily Heat Units; Rain (mm).

2.4.2. Other Soil Parameters

Soil samples were collected at the same time as the first earthworm sampling during the 2015–2016 summer season. Six soil cores were collected randomly to make a composite sample for each plot at 0–20 cm depth after removing the surface litter layer. A portion of the fresh soil for each sample was kept for the analysis of microbial biomass carbon (MBC), whilst the rest of the soil was air-dried and passed through a 2-mm sieve. The chloroform fumigation extraction procedure was used for the determination of microbial biomass carbon (MBC) as outlined in Muzangwa [14]. Soil organic carbon (SOC) was determined by dry combustion using a LECO Tru-Spec CN Analyzer, Michigan, USA. Soil pH was determined in de-ionized water at a soil: water ratio of 1:2.5 (w/v) using a glass electrode pH meter [20].

2.5. Data Analysis

Data were organized into a 2 × 4 × 2 split-split plot design and analyzed using the JMP® Release 12.0 statistical package to test the interactive effects of tillage, crop rotations, and residue management [21]. Earthworm count data were first transformed using x² + 1 to normalize the data [22]. Earthworm diversity analysis during each season was calculated using the Shannon Diversity Index. Correlation analysis was performed among the earthworm parameters—earthworm density (ED), species evenness (E), diversity index (H'), and earthworm biomass (EB) with soil and climatic parameters using the SPSS statistical package [23]. Mean separation for tests that were significantly different was performed using the least significant difference (LSD) at 5% significance level.
3. Results

3.1. Earthworm Genera and Age Proportions

The earthworm communities showed distinct seasonal patterns ($p < 0.05$), with the highest diversity during the summer 2016–2017 season (Table 3). The Shannon–Weiner diversity index was calculated for each sampling time showed the trend: summer 2016–2017 > spring 2015 > winter 2015 > summer 2015–2016 (Table 3). All three factors had a significant effect ($p < 0.05$) on genus composition in the summer of 2015–2016, winter 2016, spring 2016, and summer 2016–2017 seasons. The observed earthworm communities comprised three exotic genera belonging to the epigeic and anecic groups. The identified genera were *Lumbricus* and *Amynthas* representing the anecic group, as well as *Dendrobaena*, representing the epigeic group. During the summer of 2015–2016, no-till favored all the three genera, whilst CT favored only two except *Amynthas* (Table 4). Of the summer 2015–2016 counts, 80% of the total earthworms across treatments comprised *Dendrobaena* followed by *Lumbricus* at 12%, and lastly, *Amynthas* at 8%. The *Amynthas* genus was virtually absent, while *Lumbricus* had the highest composition in the second sampling performed in the winter of 2016. The last two samplings (spring 2016 and summer 2016–2017) showed the presence of all three genera across the treatments. *Dendrobaena* maintained greater numbers than any other genera at most of the sampling periods (Table 4).

| Season               | H’       | E       |
|----------------------|----------|---------|
| Summer 2015–2016     | 0.59 b*  | 0.54 b  |
| Winter 2016          | 0.68 b   | 0.62 b  |
| Spring 2016          | 1.04 a   | 0.95 a  |
| Summer 2016–2017     | 1.09 a   | 1.00 a  |

* Different letters under the same column represent significant differences between seasonal means at LSD = 0.05.

| Season               | CT        | No-Till  |
|----------------------|-----------|----------|
| Summer 2015–2016     | 159 b*    | 174 a    |
| *Dendrobaena*        | 38 a      | 26 b     |
| *Lumbricus*          | 0 b       | 28 a     |
| Winter 2016          | 73 b      | 135 a    |
| *Dendrobaena*        | 140 a     | 120 b    |
| *Lumbricus*          |           |          |
| Spring 2016          | 103 b     | 146 a    |
| *Dendrobaena*        | 71 a      | 74 a     |
| *Lumbricus*          | 51 b      | 100 a    |
| Summer 2016–2017     | 233 a     | 102 b    |
| *Dendrobaena*        | 216 a     | 138 b    |
| *Lumbricus*          | 166 a     | 141 b    |

* Different letters under the same genus represent significant differences between treatment means at LSD = 0.05; CT—conventional tillage.

The assessment of earthworm proportions showed the greatest percentage of mature earthworms (i.e., earthworms with a well-developed clitellum) amongst the *Amynthas* genus, even though they had
the least total counts of earthworms in the 2015–2016 summer season (Figure 1A and Table 4). During
the 2016 winter season, *Dendrobaena* and *Lumbricus* were the identified genera. Of these, 77% were
juveniles of *Dendrobaena* and 57% juveniles of *Lumbricus* (Figure 1B). Increased maturity in *Lumbricus*
and *Amynthas* was observed during spring 2016 (Figure 1C). Lastly, for the summer 2016–2017 season,
*Amynthas* had the greatest percentage of juvenility observed for all the genera (Figure 1D).

![Figure 1](image)

**Figure 1.** Genus and age proportions of earthworms sampled in summer 2015–2016 (A), winter 2016 (B),
spring 2016 (C), and summer 2016–2017 (D) at the UFH research farm.

### 3.2. Earthworm Density and Biomass

The results also showed a significant three-way interaction between tillage, crop rotations, and
residue management with respect to total density and biomass. The earthworm densities in different
sampling seasons followed the order: summer 2016–2017 > spring 2016 > summer 2015–2016 > winter
2016 (Table 5). Earthworm density was generally higher under no-till compared to CT during the first
three seasons of sampling (summer 2015–2016, winter 2016, and spring 2016). A different trend in terms
of earthworm density was observed during the summer 2016–2017 season, where CT significantly
showed improved earthworm numbers as opposed to no-till. Mature earthworms generally had high
biomass compared to juvenile earthworms due to size differences. Furthermore, the total biomass of
the earthworms increased with the season or as the earthworms matured (Table 6). The earthworm
biomass in different sampling seasons followed the order summer 2016–2017 > spring 2016 > summer
2015–2016 > winter 2016. The highest biomass was observed under residue retention irrespective of
the main factor treatment.
Table 5. Tillage × crop rotation × residue management effects on earthworm total density (counts m$^{-2}$) for summer 2015–2016, winter 2016, spring 2016, and summer 2016–2017 seasons at the UFH research farm.

|                  | CT                | No-Till           |
|------------------|-------------------|-------------------|
|                  | R−               | R+               | R−           | R+               |
| **Summer 2015–2016** |                  |                  |              |                  |
| MFM              | 19 abc*           | 21 abc           | 10 abc       | 13 bc            |
| MFS              | 15 abc            | 21 abc           | 18 bc        | 32 a             |
| MWM              | 17 abc            | 31 ab            | 27 bc        | 32 a             |
| MWS              | 19 bc             | 31 ab            | 9 c          | 33 a             |
| **Winter 2016**  |                  |                  |              |                  |
| MFM              | 16 bc             | 19 bc            | 22 bc        | 19 bc            |
| MFS              | 14 c              | 14 c             | 22 bc        | 22 bc            |
| MWM              | 27 bc             | 27 bc            | 25 bc        | 33 ab            |
| MWS              | 27 bc             | 30 abc           | 27 bc        | 46 a             |
| **Spring 2016**  |                  |                  |              |                  |
| MFM              | 15 fg             | 19 fg            | 37 cd        | 30 de            |
| MFS              | 12 fg             | 30 de            | 9 g          | 33 cd            |
| MWM              | 16 g              | 41 c             | 30 de        | 56 b             |
| MWS              | 21 ef             | 30 cde           | 11 fg        | 67 a             |
| **Summer 2016–2017** |                  |                  |              |                  |
| MFM              | 52 def            | 63 bc            | 25 i         | 27 hi            |
| MFS              | 49 efg            | 57 ab            | 30 hi        | 27 hi            |
| MWM              | 63 abc            | 60 ab            | 33 fghi      | 33 hi            |
| MWS              | 68 ab             | 71 a             | 44 de        | 41 ghi           |

* Different letters under the same treatment represent significant differences between treatment means at LSD = 0.05. MFM—maize–fallow–maize; MFS—maize–fallow–soybean; MWM—maize–wheat–maize; MWS—maize–wheat–soybean rotations. R− is residue removal; R+ is residue retention. CT—conventional tillage.

Table 6. Tillage × crop rotation × residue management effects on the ash-free dry biomass (g m$^{-2}$) for summer 2015–2016, winter 2016, spring 2016, and summer 2016–2017 seasons at the UFH research farm.

|                  | CT                | No-Till           |
|------------------|-------------------|-------------------|
|                  | R−               | R+               | R−           | R+               |
| **Summer 2015–2016** |                  |                  |              |                  |
| MFM              | 6.16 h*           | 13.00 fgh         | 28.02 cd     | 17.51 ef         |
| MFS              | 10.01 gh          | 18.53 ef          | 14.23 efg    | 31.10 c          |
| MWM              | 7.91 gh           | 14.74 efg         | 21.46 de     | 41.90 b          |
| MWS              | 8.63 gh           | 9.97 gh           | 20.51 e      | 50.93 a          |
| **Winter 2016**  |                  |                  |              |                  |
| MFM              | 26.00 fg          | 16.52 hi          | 39.13 cd     | 22.23 gh         |
| MFS              | 14.34 i           | 16.45 hi          | 24.00 fg     | 30.69 ef         |
| MWM              | 34.46 de          | 30.25 ef          | 38.27 cd     | 50.78 b          |
| MWS              | 36.47 de          | 39.38 cd          | 44.68 bc     | 59.35 a          |
| **Spring 2016**  |                  |                  |              |                  |
| MFM              | 26.12 f           | 28.93 f           | 15.65 g      | 62.83 cd         |
| MFS              | 7.69 g            | 42.67 e           | 33.10 ef     | 53.93 d          |
| MWM              | 28.61 f           | 74.61 b           | 38.61 e      | 11.63 a          |
| MWS              | 31.90 ef          | 75.06 b           | 39.32 e      | 117.82 a         |
| **Summer 2016–2017** |                  |                  |              |                  |
| MFM              | 59.16 abc         | 64.56 ab          | 29.36 e      | 26.94 e          |
| MFS              | 51.66 bcd         | 68.70 ab          | 31.81 e      | 28.11 e          |
| MWM              | 64.22 ab          | 66.97 ab          | 37.52 de     | 37.90 de         |
| MWS              | 72.68 a           | 77.10 a           | 41.86 cde    | 44.49 cde        |

* Different letters under the same treatment represent significant differences between treatment means at LSD = 0.05. MFM—maize–fallow–maize; MFS—maize–fallow–soybean; MWM—maize–wheat–maize; MWS—maize–wheat–soybean rotations. R− is residue removal; R+ is residue retention. CT—conventional tillage.
3.3. Soil Organic Carbon (SOC), Microbial Biomass Carbon (MBC), and pH

SOC percentages ranged from 1.93% under no-till in MWM rotation (residues removed) to the highest 2.25% under no-till in MFS rotation with residues retained (Table 7). The SOC values were statistically higher with residue retention than with residue removal across the treatments. MWS rotation had the greatest SOC under both CT and no-till. A significant three-way interaction between tillage, crop rotation, and residue management \((p < 0.05)\) was observed on MBC (Table 7). Tillage significantly reduced MBC levels in the soil, as higher MBC was observed under no-till (107.83 mg.kg\(^{-1}\) soil) as opposed to CT (50.09 mg.kg\(^{-1}\) soil). The same trend was observed for crop rotations, with the highest MBC under the MWS followed by MWM rotation. Residue management also showed marked differences on MBC, with the highest levels observed under residue retained than those with residues removed. The pH values ranged from 6.11 to 7.01, with a higher threshold under treatments where residues were removed.

Table 7. Tillage \(\times\) crop rotation \(\times\) residue management effects on selected soil properties at the UFH research farm.

|                | CT               | No-Till          |
|----------------|------------------|------------------|
|                | R\(^-\)        | R\(^+\)        | Mean        | R\(^-\)        | R\(^+\)        | Mean        |
| SOC (%)        |                 |                 |             |                 |                 |             |
| MFM            | 1.96 b\(^*\)    | 2.06 a\(\ldots\) | 2.01        | 1.98 b        | 2.07 a        | 2.03        |
| MFS            | 1.96 b          | 2.14 a          | 2.05        | 1.96 b        | 2.0 ab        | 1.98        |
| MWM            | 1.93 b          | 1.99 b          | 1.96        | 2.00 ab       | 2.13 a        | 2.07        |
| MWS            | 2.05 a          | 2.15 a          | 2.10        | 2.07 a        | 2.25 a        | 2.16        |
| Mean           | 2.01            | 2.06            | 2.00        | 2.00          | 2.11          |             |

| MBC (mg. kg soil\(^{-1}\)) | Crop rotation |
|----------------------------|---------------|
| MFM                        | 41.69 d       | 61.89 cd       | 51.79       | 92.25 b       | 101.81 ab     | 97.03       |
| MFS                        | 24.80 e       | 70.29 c        | 47.55       | 82.03 bc      | 117.02 ab     | 99.53       |
| MWM                        | 21.12 e       | 82.27 bc       | 51.7        | 91.28 b       | 142.15 a      | 116.72      |
| MWS                        | 28.13 e       | 70.53 c        | 63.4        | 76.25 b       | 159.83 a      | 118.04      |
| Mean                       | 28.94         | 71.25          | 85.45       | 130.2         |               |             |

| pH (H\(_2\)O)              |               |                 |             |                 |                 |             |
| MFM                        | 7.09 a         | 6.98 b          | 7.04        | 6.87 b         | 6.77 c         | 6.82        |
| MFS                        | 7.04 a         | 6.72 c          | 6.88        | 7.02 a         | 6.19 c         | 6.61        |
| MWM                        | 6.98 b         | 6.77 c          | 6.88        | 6.92 b         | 6.21 c         | 6.56        |
| MWS                        | 6.83 b         | 6.81 b          | 6.82        | 6.89 b         | 6.11 c         | 6.5         |
| Mean                       | 6.99           | 6.82           | 6.93        | 6.32           |               |             |

\(*\) Different letters under the same treatment represent significant differences between treatment means at LSD = 0.05. Mean significant differences at \(p = 0.05\); ns—not significant. UFH CA—University of Fort Hare conservation agriculture trial. SOC—soil organic carbon. MFM—maize–fallow–maize; MFS—maize–fallow–soybean; MWM—maize–wheat–maize; MWS—maize–wheat–soybean rotations. R\(^-\) is residue removal; R\(^+\) is residue retention. CT—conventional tillage.

3.4. Correlations Between Earthworm Parameters and Soil Properties

The diversity index (H') was significantly correlated with soil pH, SOC, MBC, and moisture, while species evenness (E) was only significantly correlated with soil pH and SOC (Table 8). Earthworm density (ED) was positively correlated with SOC, MBC, and soil moisture, while earthworm biomass (EB) was significantly correlated with SOC, MBC, and moisture.
Table 8. Pairwise correlation between earthworm species diversity (H'), evenness (E), density (ED), and biomass (EB) and selected abiotic and biotic parameters across the seasons and experimental factors at the UFH research farm.

|            | H'   | E    | ED   | EB   |
|------------|------|------|------|------|
| pH         | 0.68*| 0.70*| 0.19 ns | 0.12 ns |
| SOC        | 0.97*| 0.72*| 0.86*  | 0.91*  |
| MBC        | 0.94*| 0.46 ns | 0.70*  | 0.85*  |
| Temperature| 0.30 ns | −0.40 ns | 0.19 ns  | 0.47 ns  |
| Rainfall   | −0.13 ns | −0.29 ns  | 0.39 ns  | 0.49 ns  |
| Soil moisture | 0.81* | 0.35 ns | 0.79*  | 0.94*  |

ns defines correlation that is not significant and * defines correlation that is significantly different at \( p = 0.05 \).

4. Discussion

Three exotic earthworm genera were observed in the CA trial, which was set on a Haplic Cambisol in Alice within the Raymond Mhlaba municipality. The absence of indigenous earthworm genera in the CA trial site could be attributed to the fact that indigenous earthworms prefer natural undisturbed soils [24] and areas with a high density of native plant species [25]. The site at which this study was based has had no indigenous plant species for a long time (>25 years) as it has been used for field crop and forage farming. Furthermore, before trial establishment, the land had been under lucerne, while after 2012, it had been under maize, wheat, or soybean planted in rotations. This continuous cultivation could be responsible for the disappearance of indigenous earthworm species from the trial site.

The higher earthworm density observed under no-till than under CT over the first three seasons (summer, winter, and spring 2015) can be the result of a higher loss of organic matter under CT than under no-till due to accelerated oxidation of organic matter during tillage [26]. Furthermore, van Capelle et al. [27] described increased earthworm density under no-till as due to the interacting effects of reduced injuries, decreased exposure to predators at the soil surface, decreased microclimate changes, and increased availability of organic matter, providing a convenient food source in the upper soil layers. A contrasting trend was observed during the summer 2016–2017 season, where earthworm species density was greater under CT than under no-till for all three earthworm species. Ernst and Emmerling [12] observed a similar trend and suggested that the abundance of earthworms under CT in some situations is likely to be related to lower soil bulk density within the tillage zone and increased transport of organic matter below the soil surface as a result of the plough effect.

Farming systems that include the use of diversified crop rotations have been reported to positively impact earthworm density [28,29]. The observed improved earthworm densities and biomass in rotations that had a winter crop are consistent with the findings of Schmidt et al. [30]. According to Schmidt et al. [30], crop rotations involving cereal and legume crops provided food and favorable nutrition, which resulted in an increase in earthworm density even during the winter season. The findings of Schimidt et al. [30] could in part explain the greatest values of earthworm ash-free dry weight observed in the MWS in all the seasons. While the MFS had both cereal and legume crops in the rotation, the fallow period in between rendered the earthworms inactive as it appears that even under no-till (where conditions were assumed favorable), the earthworm dry weight and densities were low. The work by Schimidt et al. [30] mirrored the present study in that earthworm data in both experiments were collected after 3 years of legume–cereal rotations under no-till and CT tillage treatments.

The positive response of earthworms to residue retention irrespective of tillage management can be due to increased moisture availability. According to Du Toit [31] and Riley et al. [3], the short-term influence of residue retention is the improvement in soil moisture availability, while the long-term benefit is the improvement in organic matter content. Improved soil moisture under residue retention treatments favors earthworms’ survival and ensures a stable growth of earthworms during the seasons [32]. This input of crop residues is favorable for earthworm populations in terms of quantity,
nutritional quality, and continuity of earthworm life cycles throughout the year. This could explain the improved earthworm numbers starting from the spring to the summer 2016–2017 season under the MWM and MWS rotations that had the winter wheat crop.

The overall observed variability of earthworm densities and diversity during the four different sampling seasons could have been the result of the differences in moisture and rainfall patterns over the seasons. For instance, during the summer 2015–2016 season, there were observably high temperatures coupled with very little effective rainfall, even in the previous months before sampling. This could be the reason why the lowest earthworm density was observed in the summer 2015–2016 season. The reason for such a trend is seasonal mortality because of high temperature, which prevailed in the months before the sampling of earthworms. The situation changed in summer 2016–2017 when the amount of rainfall received in this season surpassed that which was received in the preceding seasons, creating conditions that were more conducive for earthworm growth and development. The findings of the present study are consistent with findings by Smetak et al. [33] who found that harsh environmental conditions not only limit reproduction but also the survival of adult earthworms from year to year.

The highest MBC was observed under the MWM and MWS and treatments with MFM and MFS receiving the least MBC, indicating that diversified crop rotations favored greater microbial activity. As observed earlier, these rotations also supported a greater earthworm abundance and diversity due to their ability to improve SOC. Therefore, the observed increase in microbial diversity could be attributed to the known effect of earthworms to breakdown and macerate organic matter and making it a better substrate for microbial growth. The correlations between the earthworm data and abiotic factors observed soil pH, soil moisture, SOC, and MBC as the major determinant factors of earthworm abundance measures. Dominguez and Edwards (1997) reported that environmental conditions are most likely to affect earthworm diversity through several mechanisms. A similar trend was identified by Ganihar [34], where it was observed that natural factors such as temperature, moisture, and organic matter are also important parameters affecting the diversity of earthworms.

5. Conclusions

Three exotic earthworm genera were observed from the trial and these were *Amynthas*, *Lumbricus*, and *Dendrobaena*. Tillage, crop rotations, and residue management had a significant influence on earthworm diversity and density. No-till consistently favored earthworm abundance and diversity during the first three sampling seasons, while a contrasting trend was observed during the last sampling period of the summer 2016–2017 season. Moreover, rotations, which included cereal and legume crops together with residue retention, positively influenced earthworm diversity and density irrespective of tillage management. Earthworm parameters measured in the study significantly correlated with pH, SOC, MBC, and soil moisture, suggesting that these abiotic and biotic factors are the explanatory variables for earthworm growth and development.

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