Cultivating Insights Investigating the Impact of Diverse Cropping Systems on Soil Physiochemical Parameters and Earthworm Diversity

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ABSTRACT

This research aims to assess the effects of earthworm diversity on soil physico-chemical parameters in several cropping systems, including moong-wheat, soybean-wheat, and basmati-wheat, under both conventional and organic farming practices.Location and Length of Study:The research was carried out from June 2020 to March 2021 at the MLSU (udaipur), Rajasthan, India. The four earthworm species discovered during the research period are Travoscolides chengannure, Amynthas morissia, Lampito mauritti, and Metaphire posthuma. These species are members of the Megascolicidae and Octochateidae families. Of them, Rajasthan reported Travoscolides chengannure for the first time. According to the findings, compared to conventional farming systems, organic farming systems had a higher variety of earthworms. An examination of the relationship between earthworm abundance and the physicochemical characteristics of soil in various farming systems showed that there is a positive, but non-significant, association between earthworm abundance and pH, nitrogen, and potassium levels in organic farming systems. Nitrogen, electric conductivity, and organic carbon were shown to have a strong positive connection ($p=0.01$) in the traditional agricultural system. The results of this study support the use of organic agricultural methods in place of conventional ones. By using these methods, the variety of earthworms is increased and the soil is enriched with several major and micronutrients. For long-term soil production, earthworm-friendly agricultural techniques should be used.

Keywords: Soil; physico-chemical; earthworms; cropping method.

1. INTRODUCTION

The basic characteristic of soil that influences its physical characteristics, such as water infiltration, plant nutrient absorption, soil porosity, hydraulic conductivity, water holding capacity, etc., is soil structure. Earthworm populations greatly enhance the structure of the soil via a variety of processes, including deep burrows, proper aeration of the soil, and thorough mixing of the organic leftovers with the soil particles. They actively contribute to the cycling of nutrients, making agricultural soils more productive and fertile overall. Thus, it is accurate to state that the presence or even abundance of earthworms in the soil profile is a good indicator of the health of the soil. Furthermore, they are essential for the biological breakdown of organic fuel, which in turn causes nutritional rotation. The castings of earthworms that only eat litter provide enormous quantities of plant nutrients. Because earthworm castings have higher availability of nitrogen, phosphorous, potassium, and calcium than surrounding soil, the soil is much more enriched with important nutrients.

Additionally, there is a significant amount of interference from soil management techniques with earthworm activity. Thus, any change in their activity suggests the soil's richness and condition. Additionally, some earthworms consume poisonous soil nematodes for food. These earthworm species aid in lowering the number of toxic nematodes in the soil. Researchers are very interested in using earthworms to decompose organic waste into simpler forms. The earthworms drive the cycles of energy transformation by breaking down organic materials and mineralizing nutrients that are attached to the earth, including lignocellulose. Because of the many degrading microbes in their stomachs, earthworms degrade organic things. Numerous earthworm species may be used as bioindicators to find chemical toxicity in the soil. This is a result of the earthworms' propensity to amass significant concentrations of these substances inside their tissues. Thus, the goal of the current study was to assess how earthworm diversity is related to the physico-chemical characteristics of soil in various cropping systems.

2. MATERIALS AND METHODS

2.1 Earthworm Collection and Identification

Both conventional and organic agricultural techniques were used at each research location as earthworms were hand-sorted up to 50 cm deep (25 cm x 25 cm). The earthworms were taken out of the block indicated before. Additionally, a spade was used to excavate a deeper region in order to gather the individual earthworms that burrow deeply. After being carefully cleaned with tap water, the retrieved earthworms were dried on filter paper. The earthworms were also stored in a 5% formalin solution and disinfected with 70% ethanol. Next, all of the preserved earthworms were inspected under a stereomicroscope for morphoanatomical traits like the total number of segments, prostomium shape, clitellum position and type, spermathecae position and number, male pore position, and total length (in centimeters) using a measuring scale. We looked at these exterior features by using a microscope to examine samples of earthworms. Eventually, the number of segments from prostomium to anus was counted using a pointed needle.

2.2 Earthworms' Molecular Characterization

Four distinct conventional and organic cropping systems—basmati-wheat, basmatic-hickpea, soybean-wheat, and moong-wheat—were used to gather various types of earthworms. The earthworm species that were gathered were kept in 100% ethanol. DNA was extracted, and its amplification for the mtCO-I gene was done.

Using a modified CTAB approach, the complete genomic DNA of earthworms was extracted, and the amount was quantified using a NanoDrop Spectrophotometer.

Furthermore, a 0.8% agarose gel was used to assess the quality. Once again, the mtCO-I gene was subjected to PCR using "universal primers, namely" LCO 1490 (5′- GCTCAACAAATCATAAAGATATTGG-3′) and HCO 2198 (5′- TTTCAGGAAACGTGACCAA AAAATCA -3′).

The following conditions were used for the PCR amplifications in a VeritiTM 96-Well Thermal Cycler (Applied Biosystem, USA): three minutes of denaturation at 94°C, 38 cycles at 94°C for 30 seconds, annealing at 52°C for 45 seconds, and one minute at 72°C. Ten minutes at 72°C were then held at 4°C for the ultimate elongation step. Resolved on 1.5% agarose gel, a single 750 bp distinct PCR amplicon band was seen. In accordance with the manufacturer's instructions, the "barcode fragment"/mtDNA COI 3' fragment gene PCR amplicon was eluted and purified using NucleoSpin Gel and PCR Clean-up kit. A forward and a reverse primer were used, respectively, in the PCR amplicon sequencing procedure for the LCO1490 forward and HCO 2198 reverse DNA sequences. In addition, BDT v3.1 was used on an ABI 3730xl Genetic Analyzer in conjunction with a cycle sequencing kit to determine the consensus sequence for the barcode fragment gene. Lastly, the NCBIBasic Local Alignment Search Tool database was used by taking advantage of the insect DNA barcode fragment gene sequence. The first 10 sequences were chosen and aligned using ClustalW multiple alignment, a multiple alignment software application, based on maximal identity score. Using MEGAX, a distance matrix was created and a phylogenetic tree was built.

2.3 Soil Physico-Chemical Analysis

Temperature, moisture content, and other physicochemical characteristics of the soil, including N, P, K, OC, EC, and pH, were analyzed for both conventional and organic agricultural methods. The soil samples were burned at 550 °C in a muffler furnace, and the organic carbon content was calculated using the 1934 Walkely and Black technique. Kjeldahl assembly was used to determine the amount of nitrogen in the soil. According to Subbiah and Asija (1956), the soil sample was first digested with concentrated H_2SO_4, after which it was put through the Kjeldahl assembly. Finally, the titration was completed using 0.01 N HCl. Additionally, a spectrophotometer was used to measure the phosphorus content of the soil. It included the use of nitric acid and perchloric acid in a 1:4 ratio to digest the soil, respectively. Then, Olsen's approach was used to determine phosphorus using a colorimeter. The following is an evaluation of the soil's moisture content and temperature:

Soil temperature: The temperature of the soil in the field was measured using a device called a digital thermometer. The temperature was measured after the thermometer was placed straight into the ground and left there for a minute. Every month after each crop was sown, the temperature of the soil was measured.

Soil moisture: Through the use of gravimetric analysis, soil moisture was measured. This procedure included sieving a new soil sample and determining its core weight. After oven drying, it was weighed again.

2.4 Statistical Analysis

Using SPSS version 16.0, the t-test analysis was performed to examine the soil parameters of the two chosen farming systems, namely the organic and conventional farming systems, at $p \leq 0.01$ and 0.001.

3. RESULTS AND DISCUSSION

Earthworm molecular characterization: Using a "barcode fragment" or mtDNA COI 3' fragment based on DNA barcoding technique, earthworms are identified.

The earthworm samples 1 (E1) and 2 (E2) were determined to be Metaphire posthuma based on sequence homology and phylogenetic analysis, whereas the 3 (E3) was determined to be

Travoscolideschengannure (Fig. 1).

The NCBI-Basic Local Alignment Search Tool database was used to search the insect DNA barcode fragment gene sequence. The first 10 sequences were chosen and aligned using the ClustalW multiple alignment software tool based on their highest identity score.
Using MEGAX, a distance matrix was produced and a phylogenetic tree (Fig. 2) was built.

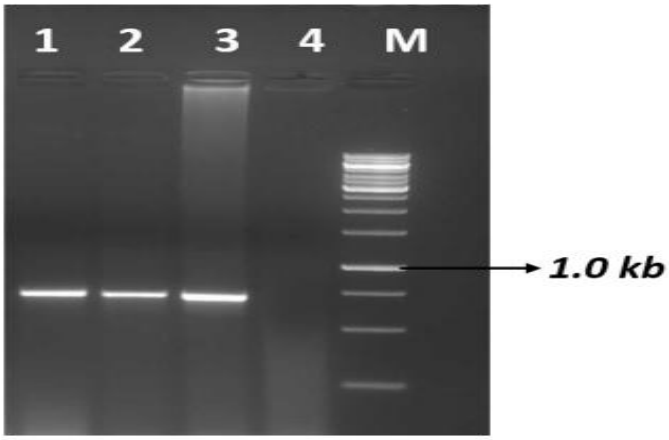

Fig. 1. Earthworm gDNA sample agarose (1.5%) gel electrophoresis of PCR using LCO1490 and HCO2198 primers

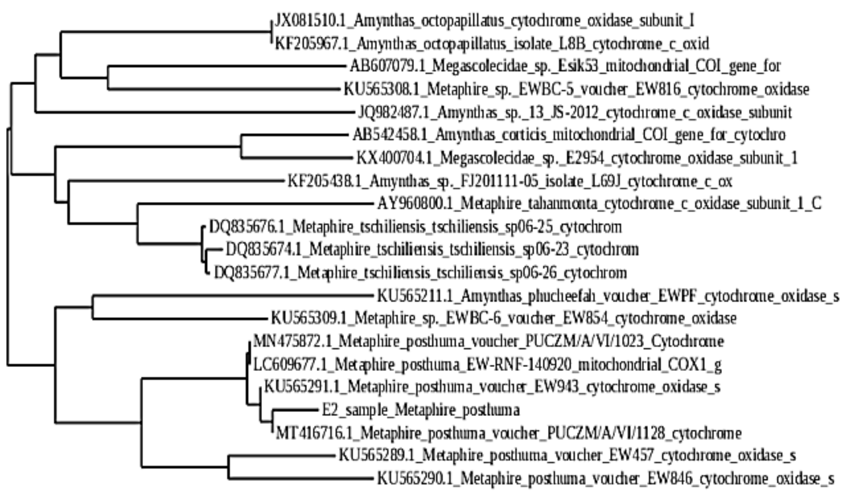

Fig. 2. Evolutionary relationships of earthworm E2 sample with other taxa

The Megascolecidae family is the most varied in Punjab, Haryana, and Himachal Pradesh, according to Panjgotra and Ahmed et al. While the Octochaetidae family has a lumbricine arrangement with eight setae tightly coupled around the segment, the Megascolecidae family has a perichaetine structure with setae placed in a ring around the segment. The methods in the keys that matched the setae arrangement were used to identify the species of earthworm. Species and families have different spermathecae pores. The Octochaetidae family only contains one to two spermathecae pores, while the Megascolecidae family has two to four. In all known earthworm species belonging to the Megascolecidae family, with the exception of L. mauritii, whose clitellum was situated at segments 14–17, the clitellum was annular. In contrast, the clitellum location was found in segments 13–17 in animals belonging to the Octochaetidae family. The male pore ranged from 17 to 18 in the Octochaetidae family and was found at segment 18 in the Megascolecidae family. Similar physical characteristics have been noted by many researchers in earthworm specimens belonging to the families Megascolecidae and Octochaetidae.

3.1 Soil's Physico-Chemical Properties

In soils with various cropping patterns, the statistical analysis of pH, electrical conductivity (EC), nitrogen (N), phosphorus (P), potassium (K), and organic carbon (OC) was provided. In organic soils, the pH was found to be between 7.12 and 7.80, with an average of 7.5. The pH range in ordinary soils was 7.11–7.56. In contrast, EC (mS) varied between 0.19 and 0.22 in conventional farming systems and between 0.22-0.36 in organic agricultural systems. The average EC concentration in conventional and organic fields was 0.36 and 0.19, respectively. In organic fields, the OC varied from 0.54 to 0.63, whereas in conventional soils, it ranged from 0.41 to 0.46. On the other hand, organic soils had a greater nitrogen content (362.40 g/kg) than conventional soils (339.40 g/kg). Additionally, it was shown by Hackenberger and Hackenberger that the physico-chemical characteristics of the soil, such as organic matter and climate type, affect earthworm biodiversity. Additionally, Singh et al. used principal component analysis to show that the distribution of earthworm communities is closely correlated with soil physico-chemical parameters as pH, moisture content, and organic carbon. Organic farming had the highest pH in B-W (7.7±0.05), followed by conventional farming (7.1±0.09), but there was no significant difference in pH between the two types of farming for B-W. Organic farming had the greatest pH in B-C (7.6±0.03), while conventional farming had the lowest pH (7.2±0.05). However, there was no discernible difference in B-C between the two types of farming. Organic farming had the highest pH in S-W (7.8±0.05), followed by conventional farming (7.1±0.05), and this difference was determined to be significant. Significantly, the greatest pH in M-W was found in organic farming (7.1±0.05), followed by conventional farming (7.3±0.03).

Organic farming had the highest EC in B-W (0.26±0.005), while conventional farming had the second-highest EC (0.22±0.005). There was a significant difference between the two types of farming for B-W. Organic farming had the greatest EC in B-C (0.22±0.005), while conventional farming had the lowest EC (0.21±0.005). There was no statistically significant difference between the two types of farming for B-C.

Organic farming had the greatest EC in S-W (0.22±0.005), while conventional farming had the lowest EC (0.19±0.05), both of which differed considerably. Organic farming had the greatest EC in M-W

(0.36±0.005), while conventional farming had the lowest EC (0.20±0.005). There was a substantial difference in M-W between the two types of farming.

Organic farming had the greatest OC in B-W (0.63±0.005), while conventional farming had the lowest OC (0.46±0.005). A significant difference was identified between the two types of farming for B-W. Organic farming had the greatest OC in B-C (0.54±0.005), whereas conventional farming had the lowest OC (0.44±0.005). No discernible variation was seen. Organic farming had the greatest OC in S-W (0.59±0.005), followed by conventional farming (0.41±0.005). This difference was significant for SW. Organic farming had the greatest OC in M-W (0.62±0.005), with conventional farming coming in second (0.0±0.005). This difference was statistically significant. Furthermore, Chan and Barchia's research revealed that the most important and significant factor influencing both the quantity and dispersion of earthworm communities in a given location is the organic carbon (OC) content of the soil.

The study revealed a substantial difference in nitrogen levels between organic and conventional farming practices in B-W, with organic farming recording the highest N levels at 356.7±2.1, and conventional farming coming in second at 339.4±3.25). In B-C, organic farming had the greatest nitrogen content (362.4±1.35), followed by conventional farming (317.2±1.47), but there was no discernible difference in B-C's nitrogen content between the two types of farming. Organic farming produced the largest amount of nitrogen (N) in S-W (360.6±0.7), with conventional farming coming in second (314.8±1.49). There was a considerable difference in N between the two types of farming in S-W. The greatest N in M-W was reported in organic farming (324.1-21.9), followed by conventional farming (315.6-7.7). There was a substantial difference in M-W between the two types of farming.

Organic farming had the greatest P in B-W (50.3±0.7), with conventional farming coming in second (45.5±1.65).and was found to differ significantly between conventional and organic farming for B-W. Organic farming had the highest P in B-C (44.2±0.7), followed by conventional farming (41.4±0.56), but there was no discernible difference between the two types of farming in B-C. The greatest P in S-W was reported in organic farming (48.1±1.46), followed by conventional farming (42.6±1.6). There was a substantial difference in S-W farming between the two types of farming. Organic farming had the highest P in M-W (50±0.75), while conventional farming came in second (44.2±0.81). There was a substantial difference between the two types of farming in M-W.

The greatest K in B-W was discovered in organic farming (151.8±0.7), which was followed by conventional farming (145.4±2.33). There was also a substantial difference in B-W farming between the two types of farming. Organic farming in British Columbia had the highest K value (152.3±1.19), while conventional farming came in second (145.4±2.33). However, there was no discernible difference between B-C's organic and conventional agricultural practices. Organic farming in S-W recorded the highest K value at 143±1.95, with conventional farming coming in second at 137.3±1.51. The difference between S-W's organic and conventional farming was determined to be statistically significant. The M-W K-value was found to be significantly different between organic and conventional farming, with organic farming having the highest K value (151.8±0.7), followed by conventional farming (144.9±0.79).

Earthworm abundance was correlated with soil parameters in both conventional and organic farming systems. The results showed that the earthworm abundance in the organic farming system had a moderate positive correlation (r = 0.46, p = 0.13) with N, a marginal negative correlation (r = -0.21, p = 0.51) with P, and a moderate positive correlation (r = 0.56, p = 0.05) with K. This correlation was also found between the earthworm abundance and the pH of the soil, EC, and very marginal negative correlation (r = -0.17, p = 0.59). The earthworm abundance for the conventional farming system demonstrated the following: a very nominal positive correlation (r = 0.1, p = 0.75); a strong positive correlation that was statistically significant (r = 0.73, p≤0.01) with EC; a strong positive correlation that was statistically significant (r = 0.808, p≤0.001) with OC; a strong positive correlation that is statistically significant (r = 0.81, p≤0.001) with N; and an extremely nominal positive correlation (r = 0.1, p = 0.75 with K).

Table 1. Correlational analysis of earthworm abundance with the soil parameters in different farming systems

Cropping System	pH	EC	OC	N	P	K
Organic						
B-W Mean	7.7	0.26	0.63	356.7	50.3	157.8
S.E.	0.15	0.03	0.02	9.02	1.4	3.06
B-C Mean	7.6	0.22	0.54	362.4	44.2	152.3
S.E.	0.18	0.04	0.02	10.8	1.4	2.61
S-W Mean	7.8	0.22	0.59	360.6	48.2	143
S.E.	0.2	0.04	0.08	9.16	1.1	2.27
M-W Mean	7.1	0.36	0.62	324.1	50	151.8
S.E.	0.02	0.04	0.04	5.68	2.1	3.76
Conventional						
B-W Mean	7.1	0.22	0.46	339.4	45.5	145.4
S.E.	0.09	0	0	6.33	0.89	2.34
B-C Mean	7.2	0.21	0.44	320.1	41.4	136.8
S.E.	0.1	0.005	0.005	1.43	0.7	2.26
S-W Mean	7.1	0.19	0.41	316.9	42.6	137.3
S.E.	0.14	0	0	1.16	0.56	2.68
M-W Mean	7.5	0.2	0.42	311.5	44.2	144.9
S.E.	0.1	0.07	0.14	110.13	15.6	2.22
R	0.318	-0.388	-0.171	0.462	-0.21	0.562
P	0.312	0.211	0.594	0.13	0.51	0.056

Table 2. Analysis of soil parameters of organic and conventional farming systems

	Mean	SD	T	P
	Organic	Conventional		
pH	7.51±0.08	7.19±0.04	0.29	0.13
EC	0.265±0.017	0.205±0.004	0.604	0.014
OC	0.595±0.01	0.43±0.006	0.037	0.021
N	350.9±4.07	320.7±2.9	16.27	11.6
P	48.17±0.84	43.42±0.71	2.91	2.46
K	151.22±1.73	141.1±1.47	6.002	5.11

Table 3. Soil Temperature and moisture of different crop systems in both organic and conventional cropping system

Cropping Systems	Soil temperature (°C)	Soil moisture (%)
Organic basmati	23.9	37
Organic soybean	23.5	18.9
Organic moong	23.7	19.2
Organic wheat	21.8	19.3
Organic chickpea	23.1	16.4
Conventional wheat	21.2	18
Conventional chickpea	22.1	16
Conventional basmati	23.5	37.5
Conventional soybean	23.1	18.2
Conventional moong	23.5	17.5

4. CONCLUSION

The organic basmati crop in this research had the greatest soil temperature of all of the organic crops. The investigation of the relationship between earthworm abundance and soil physico-chemical parameters in various farming systems found that there was a positive, but non-significant, association between earthworm abundance and pH, nitrogen, and potassium levels in organic farming systems. Thus, it can be said that earthworm variety and soil health are better served by organic agricultural practices.

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