



# Investigating the impacts of agricultural land use on soil earthworm communities: A case study of northern Zagros Mountains of Iran

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## ABSTRACT

Earthworms play a crucial role in the invertebrate community of soil by contributing to the belowground biomass and biogeochemical cycle. Environmental stresses, such as human activities and land use changes, have been found to negatively affect their abundance and diversity. To investigate the impact of agricultural land use and pastures on earthworms' genetic diversity in the Northern Zagros Mountains, we used COI molecular marker and DNA barcoding approaches. We collected earthworm specimens from four farmland sites and six pastures and assessed the abundance and species composition of earthworm communities across the two land uses using quadrat sampling. Using the barcoding method, we identified 13 molecular operational taxonomic units (MOTUs) among the captured earthworms. Our results showed that the number of total MOTUs, density, and earthworm communities differed significantly between the two land uses. We also found that pastures had more abundant earthworms, while farmlands had greater diversity. The diversity of OTUs in the Lumbricidae family was dominant in the agricultural system. Overall, the population of invasive earthworm species in cultivation systems is influenced by chemical inputs and organic materials from plant residues, cover crops, manure, or organic fertilizers. Given the rapid rate of land use change worldwide, especially in Iran, it is crucial to understand the impact of disturbances on earthworms.

## 1. Introduction

A major part of terrestrial ecosystems exists in soil [1,2]. Soil habitat is very rich compared to the upper layers, in terms of Biological Diversity per unit [2,3]. Over the past decades, the research has intensified on ecosystem biodiversity and the functioning relationships in soil, improving our knowledge of sustainable soil biodiversity administration [4].

Anthropogenic disturbance can cause significant soil biodiversity loss in many ecosystems around the world [5]. In particular, changes in land use and intensification can significantly impact soil use efficiency due to decreased fertility, increased erosion, and loss

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of organic matter and nutrients [6–8]. It has a significant impact on soil biology and ecology, influencing interactions both above and below ground on a large scale [9]. Land use practices are related to disturbance and strongly affected the biodiversity of terrestrial communities and ecosystems, particularly the abundance and richness of soil invertebrates [10–13].

Several studies investigated the impact of land use management on biodiversity of earthworms that play a crucial role in the functions of soil [14]. Also, they are one of the most valuable indicators of soil health and a vital component of the soil fauna [15–17]. Earthworms can have an impact on the mechanism of structure, soil texture and porosity [18–20]. In addition, earthworms have a direct effect on flood regulation, climate and water filtration, and an ecological functions in remediation [21]. They contribute in soil nutrients dynamics and cycling through their casting process [22,23]. They also can live and breed in soils contaminated with heavy metals [24], as well as store heavy metals in their body [25]. Earthworms mix soil layers and bind the soil by migration at very slow rates. Also, earthworm coprolites can be of great use such as forming an organic mineral layer on the topsoil [26]. Earthworms can be categorized into three groups based on their feeding habits on different food resources: epigeic, anecic, and endogeic. Endogeic earthworms are geophagous species that mostly reside in the soil layer. They create horizontal burrows by consuming soil, and are commonly found in the soil layer [27].

Among all the fauna on agricultural land, earthworms are usually the most visible group, making up half to three quarters of the total weight. In this regard, earthworms are of great importance since they are part of the soil macrofauna and play an essential part as biotic indicators of intensified agriculture [28,29]. Van Groenigen et al. (2014) demonstrated that earthworms have a positive effect on plant growth by nitrogen mineralization, and reduce the farmer's need for applying nitrogen fertilizer [30].

Earthworms play a significant role in soil ecosystems. However, studying them presents many challenges. The morphological method of identification is time-consuming and requires specialists to identify only adult forms. Moreover, earthworms exhibit a variety of morphological and anatomical features, which vary within taxa and can overlap across them. This makes their identification difficult in most life stages [31]. DNA barcoding is a useful method that has been widely used for studying biodiversity [32–36]. It is a powerful tool for identifying earthworms accurately using standard molecular protocols [32,37,38]. This method is a novel way to solve taxonomic problems with species discrimination earthworms identifying new taxa and juveniles, and detecting of cryptic diversity [35,39]. In spite of the extensive effort invested in submitting data to NCBI's GenBank library and the Barcode of Life Database (BOLD), there are still substantial gaps in these genetic databases concerning some animal groups and geographical regions [40,41]. Due to low sequence coverage in databases, identifying Oligochaete specimens is also challenging [42].

Afshar et al. (2010) found that in Iran, pastures were frequently replaced by dryland farming [43]. The previous studies on land use in Iran mostly investigated the impact of this alternation on soil physical, chemical and hydraulic properties [44]), and earthworm communities poorly investigated [45]. Although Iran has a diverse earthworm population, there is limited knowledge on identifying species based on physiological and morphological characteristics. Some studies have been conducted on earthworms in Iran, focusing only on their identification through morphology, but in rare cases using genetic datasets [46–48]. Having a comprehensive list of species is a practical tool to evaluate the biodiversity and impact of land uses. Therefore, this study employed molecular approaches to identify soil earthworm biodiversity. The study then compared the density and diversity of soil earthworm communities between farms and pastures to assess the impact of land use in the northern part of the Zagros Mountain of Iran.

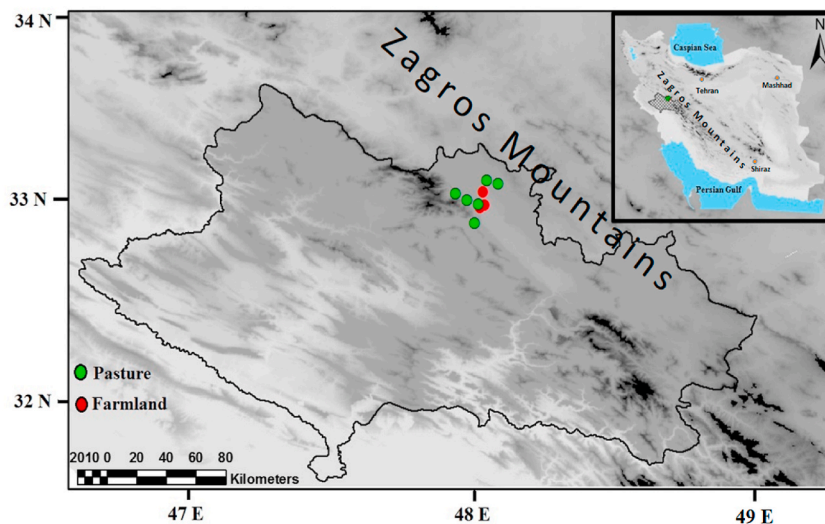


Fig. 1. Maps of the study area and sampling locations; the pasture and crop sites showed by green and red color, respectively.

## 2. Materials and methods

### 2.1. Study site

Iran possesses a unique position, which has resulted in a diverse ecosystem within the country [49]. The Zagros Mountain range stretches over 1500 km from western to southwestern Iran. This range connects the Anatolian mountain belt to the Strait of Hormuz, starting from the eastern part of Turkey, where it is located in the Makran subduction zone. Geographically, the Zagros Mountains are divided into two regions: the North Zagros in the west and the Central Zagros in the east, which are separated by the Kazerun fault sliding system that runs from north to south [50].

The Zagros Mountains have varying elevations ranging from 2000 to more than 4000 m above sea level at their peaks. The weather in the mountains differs from the lowlands to the west and east. Based on data from meteorological stations, the region has a semi-humid climate that features hot summers and relatively cold winters. The average annual maximum temperature is 21.9° Celsius, while the minimum annual temperature is 8.2° Celsius. The area experiences an annual rainfall of 456.2 mm. The dry period lasts for 154 days, starting at the beginning of June and ending at the beginning of November.

There is not much information available on land use assessment in this region. However, research conducted in the north of the Zagros Mountains suggests that a greater proportion of agricultural land will be used in the west. From the west to the east, natural land use patches will decrease, and artificial land uses will increase [51]. Over the past few decades, human construction and development have led to the creation of a new type of land use in towns and villages across the region. This type of land use requires a multi-purpose management approach that incorporates agroforestry systems, and it also encourages greater participation from local communities in this field [52].

### 2.2. Earthworm sampling

Field data were obtained of the Lorestan province in Western of Iran (33.84° - 33.9° N; 48.72° - 48.6° E) (Fig. 1). During our research,

**Table 1**

The data set of earthworms used in this study includes code ID, MOTU (obtained from COI datasets), scientific name, family and accession number.

ID code	MOTU	Scientific name	Family	Accession number
ES1695	A	<i>Helodrilus</i> sp.	Lumbricidae	OR475032
ES1717	A	<i>Helodrilus</i> sp.	Lumbricidae	OR475031
ES1744	B	<i>Metaphire</i> sp.	Megascolecidae	OR475048
ES1714	C	<i>Aporrectodea rosea</i>	Lumbricidae	OR475059
ES1715	C	<i>Aporrectodea rosea</i>	Lumbricidae	OR475058
ES1730	D	<i>Aporrectodea rosea</i>	Lumbricidae	OR475055
ES1751	E	<i>Aporrectodea caliginosa</i>	Lumbricidae	OR475030
ES1710	E	<i>Aporrectodea caliginosa</i>	Lumbricidae	OR475060
ES1750	E	<i>Aporrectodea caliginosa</i>	Lumbricidae	OR475051
ES1737	E	<i>Aporrectodea caliginosa</i>	Lumbricidae	OR475049
ES1719	E	<i>Aporrectodea caliginosa</i>	Lumbricidae	OR475038
ES1748	E	<i>Aporrectodea caliginosa</i>	Lumbricidae	OR475046
ES1722	E	<i>Aporrectodea caliginosa</i>	Lumbricidae	OR475047
ES1723	E	<i>Aporrectodea caliginosa</i>	Lumbricidae	OR475056
ES1721	E	<i>Aporrectodea caliginosa</i>	Lumbricidae	OR475057
ES1711	E	<i>Aporrectodea caliginosa</i>	Lumbricidae	OR475041
ES1752	E	<i>Aporrectodea caliginosa</i>	Lumbricidae	OR475061
ES1720	E	<i>Aporrectodea caliginosa</i>	Lumbricidae	OR475037
ES1743	F	<i>Bimastos</i> sp.	Lumbricidae	OR475025
ES1742	F	<i>Bimastos</i> sp.	Lumbricidae	OR475034
ES1712	G	<i>Bimastos</i> sp.	Lumbricidae	OR475040
ES1749	H	<i>Aporrectodea rosea</i>	Lumbricidae	OR475045
ES1713	I	<i>Aporrectodea rosea</i>	Lumbricidae	OR475039
ES1716	I	<i>Aporrectodea rosea</i>	Lumbricidae	OR475026
ES1726	J	<i>Esenia nordenskioldi</i>	Lumbricidae	OR475044
ES1738	K	<i>Aporrectodea trapezoides</i>	Lumbricidae	OR475035
ES1735	K	<i>Aporrectodea trapezoides</i>	Lumbricidae	OR475036
ES1747	K	<i>Aporrectodea trapezoides</i>	Lumbricidae	OR475033
ES1753	L	<i>Allolobophora</i> sp.	Lumbricidae	OR475029
ES1731	L	<i>Allolobophora</i> sp.	Lumbricidae	OR475054
ES1733	L	<i>Allolobophora</i> sp.	Lumbricidae	OR475052
ES1736	L	<i>Allolobophora</i> sp.	Lumbricidae	OR475050
ES1727	L	<i>Allolobophora</i> sp.	Lumbricidae	OR475062
ES1740	M	<i>Aporrectodea jasseyensis</i>	Lumbricidae	OR475042
ES1728	M	<i>Aporrectodea jasseyensis</i>	Lumbricidae	OR475027
ES1732	M	<i>Aporrectodea jasseyensis</i>	Lumbricidae	OR475053
ES1729	M	<i>Aporrectodea jasseyensis</i>	Lumbricidae	OR475043
ES1725	M	<i>Aporrectodea jasseyensis</i>	Lumbricidae	OR475027

we chose four agricultural lands that were used to cultivate sugar beet, alfalfa, clover and wheat crops, and we also selected six pastures as control. The samplings were conducted during autumn of 2018 and spring of 2019. During the initial stage of surveying earthworms in the field, we used species accumulation curves to estimate the number of species in a specific area. We discovered that the curve became stable after using five quadrates (measuring 40 cm by 40 cm). Therefore, we dug five quadrates (40 cm × 40 cm with 0.5 m depth) for examination in each site [53].

### 2.3. Soil physico-chemical properties

The soils samples randomly taken using in the middle each experimental quadrat separately with a spade. Then, they air-dried at room temperature and passed through a 2 mm sieve. Following that, the soil samples of the five quadrats within all sites were pooled together (around 700 gr per site). Soil texture was measured by hydrometric method [54] following that, other soil properties i.e. pH (pH meter - ELELA model; [55]), carbon (Walkey and Black method; [56]), total nitrogen (Kjeldahl method; [57]) were measured using standard protocols. To identify concentrations of P, Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, Na<sup>+</sup> and Al<sup>3+</sup> extracted soil chemical elements the Inductively Coupled Plasma Method (PerkinElmer ICP-OES; Zarazma Mineral Studies Company, Iran) were used.

### 2.4. Identification of earthworms

#### 2.4.1. Morphological sorting

We undertook four steps to prepare, collect, process, and store/transport the sample for testing. For long-term storage, 200 specimens were preserved in 96 % ethanol and stored at -40 °C. Using a stereo microscope, the individuals were identified based on available morphological keys [58,59].

#### 2.4.2. DNA extraction and sequencing

After sorting sample by morphological characteristics, each different morphospecies (N = 38) were selected for genetic barcoding. The genomic DNA was extracted by using salt method [60] from 5 to 10 mg of fresh or frozen solid tissues. Since the purpose of this study was to evaluate the existing biodiversity of soil oligochaetes, the mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene was utilized for molecular experiments. The primers used in the present study were as follows: LCO1490 (forward), 50 TGTAAACGACGGCCAGTGGTCAACAAATCATAAAGATATTGG- 30; HCO2198 (reverse), 50-CAGGAAACAGCTATGACTAACTTCAGGGTGACCAAAAAATCA- 30 [61]. The polymerase chain reactions (PCRs) amplification of the COI gene were done under condition 10.5 µL of nuclease-free water, 12.5 µL of Master Mix Red PCR buffer (Ampliqon), 0.5 µL of each primer, and 1 µL of DNA, the final reaction volume was 25 µL. All PCR experiments were conducted either on the MJ-Mini (48-well; Bio-Rad) or T100 (96-well; Bio-Rad, Hercules, CA, USA) thermal cyclers, started with initial denaturation at 95 °C for 10 min, followed by 34 cycles of 94 °C for 45 S, 48 °C for 45 S and 72 °C for 1 min, with a final extension at 72 °C for 6 min [62,63]. Using the 1.5 % agarose gel electrophoresis, the amplified PCR products were visualized. The successfully amplified PCR samples were then sent to Niagenenoor Company for sequencing and purification ([www.niagenenoor.com](http://www.niagenenoor.com)). COI sequences were obtained (38 individuals) from both farmlands and pastures, were edited for low quality base calls and possible sequencing errors was conducted by Geneious prime v.2021.0.0 (<http://www.geneious.com>; [64]). All newly obtained sequences were deposited to GenBank (Table 1).

#### 2.4.3. Genetic analyses

The edited sequences were searched as query in NCBI (<https://blast.ncbi.nlm.nih.gov/>) by Standard Nucleotide Basic Local Alignment Search Tool (BLASTN) [65] and Barcode of Life Data Systems (BOLD) ([66]; <https://www.boldsystems.org/>) to determine their taxonomic identities (at genus to species level).

Alignments were carried out MAFFT v. 7.036 [67] (<https://mafft.cbrc.jp/>; algorithm: Auto; scoring matrix: 200Pam/k = 2; Gap open penalty: 1.53). Under Akaike's information criterion [68] the best nucleotide substitution for the COI sequence dataset was estimated using Mrmodeltest v. 2.4 [69]. A maximum likelihood (ML) analysis was estimated using IQ-Tree v.1.6.12 [70] with 1000 bootstrap pseudoreplicates, the confidence of branch supports was determined through the ultrafast Bootstrap (UFB) approach [71]. Using MrByes v.3.2.7 [72] bayesian inference was carried out. Four chains were run in MrBayes for 3 million generations and 0.01 sampling frequency. As burn-in, 10 % of the posterior samples were cast aside. Eventually, using Tracer v.1.6 convergence to the stationary distribution was measured [73]. Uncorrected genetic distances were calculated within the dataset using MEGA X [74].

To infer hypothetical candidate species, two widely-used statistical methods of species delimitation were employed (automatic barcode gap discovery (ABGD; [75]) and Bayesian implementation of the Poisson tree processes (bPTP; [76]). ABGD is a fast method for inferring 'barcode gap' across the dataset. The ABGD web interface (online web application; available at: <http://www.wabi.snv.jussieu.fr/public/abgd/>) was used for delimiting species with the K2P model. The analysis evaluated for default values of the prior limit to intraspecific diversity (P) between 0.001 and 0.100, except X, a proxy for the minimum gap width, which was set to 1.0. bPTP infers species boundaries via the number of substitutions.

### 2.5. Statistical analyses

The statistical analyses were conducted using R v. 4.0.3 [77]. The analyses were done for MOTUs (i.e. Molecular Operational Taxonomic Units) that obtained after genetic analysis. Two-way analysis of variance (two-way ANOVA) followed by two-sided Tukey HSD tests was conducted to assess the effects of land use (farmland vs. pasture) and types of cultivation on total MOTUs diversity. We

performed an ANOVA in R using the aov function. The assumptions of homogeneity of variances (Levene’s test,  $p > 0.05$ ) and normality of residuals were tested (Shapiro–Wilk test,  $p > 0.05$ ) using R package car v. 1.2–7 [78].

The betapart package [79] in R was used to examine dissimilarities between the assemblages of earthworms of farmland and pasture using the Jaccard dissimilarity index. The dissimilarities were estimated using  $\beta$ -diversity and phylogenetic  $\beta$ -diversity approaches which measure how species (MOTUs) compositions and phylogenetic distances (branch lengths) between samples change across space, respectively [80]. It provides a theoretical framework for the partitioning of total dissimilarity into nestedness (species gains or losses) or turnover components (species replacement) [81].

A multivariate approach, permutational multivariate analysis of variance (PERMANOVA), was applied to assess the spatial compositional differences in earthworms’ compositions. Permutations of residuals ( $n = 9999$ ) were performed under the reduced model, following the same factorial design as univariate analysis and using the Sorensen similarity matrices via the vegan package. In the case of significant differences, the SIMPER routine was applied to compute the proportional contribution of each MOTUs by the observed pairwise dissimilarity in composition.

Direct ordination methods (i.e. CCA) were utilized to explore the relationship between earthworms’ diversity and environmental variables at whole study area and farmland areas using vegan v. 1.17–8, ggplot2 v. 1.0.0. and ggrid v. 1.0.0 [82].

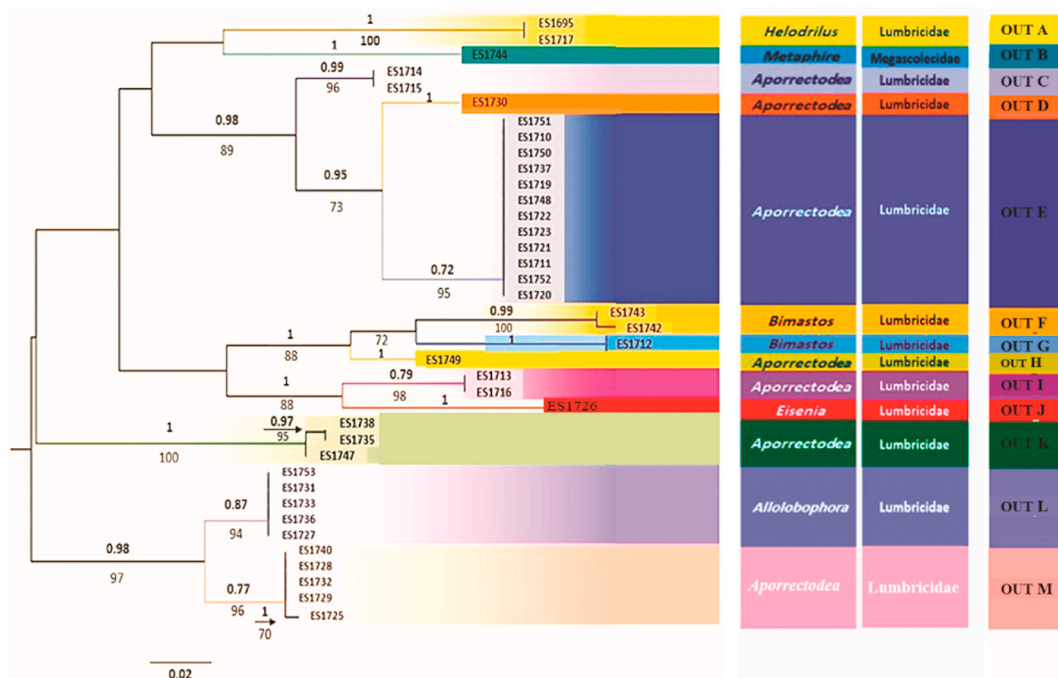
### 3. Results

#### 3.1. Identifying earthworm MOTUs

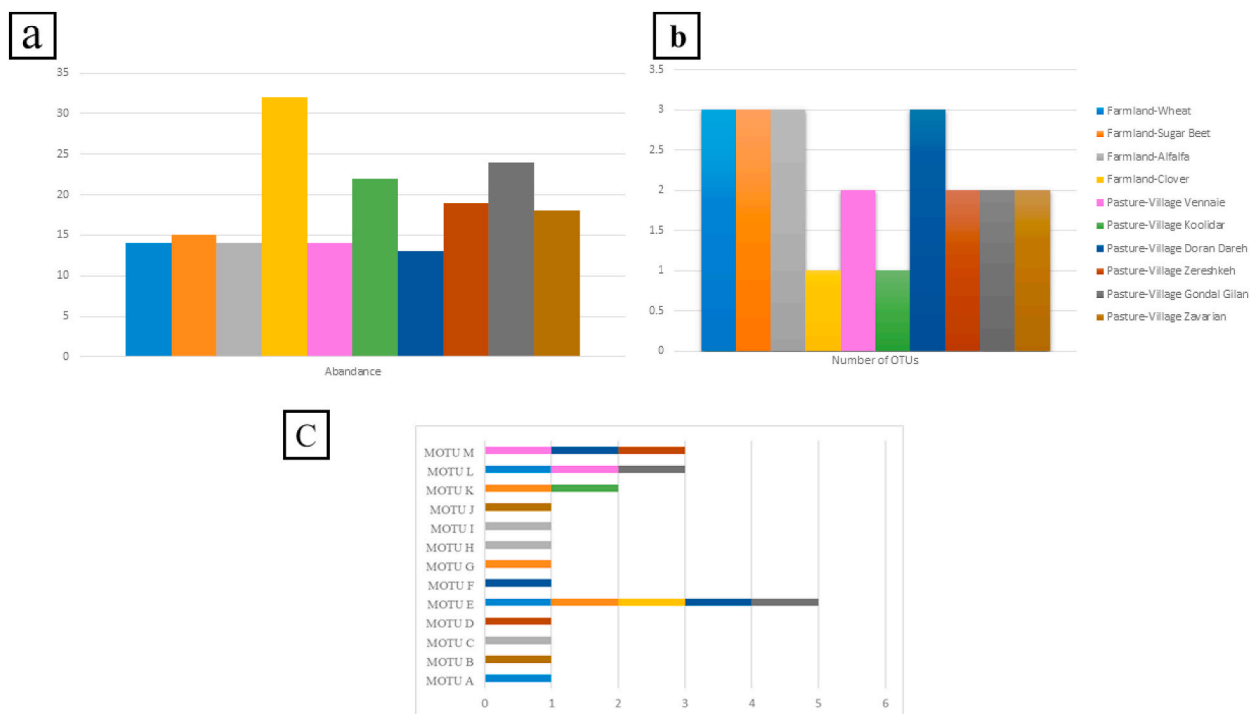
All specimens (38 sequences) could be assigned to family and genus using BLAST tools. Due to the lack of a comprehensive dataset in blast tools, we were unable to identify all of the sequences to the species level. The COI dataset revealed 13 clades in the phylogenetic tree (Fig. 2). Species delimitation with bPTP resulted in 13 putative species for the barcoding dataset. Most of them were supported with high value ( $>80\%$ ). The ABGD analysis yielded 13 hypothetical species using about 3.5 intraspecific divergence (table A. 1). The genetic distances showed the divergence between 13 MOTUs are about 5–19% (table A. 1). Overall, the combination of our findings defined 13 lineages (MOTUs) within our dataset.

#### 3.2. MOTUs diversity

In table A. 2, the distribution of MOTUs within different land uses is shown. It shows more MOTUs were recognized in farmlands (except Village Doran Dareh; Fig. 3b) than in pastures, but there were more MOTUs abundance in pastures (Fig. 3a). In addition, among farmlands, land covers with clover had higher abundance but less diversity (Fig. 3b). Moreover, there are several shared



**Fig. 2.** The phylogenetic tree of earthworms and MOTUs across location. The lineages and taxonomy identification (genus and family). The topology of BI and ML trees is the same, therefore only ML tree show and values of above and below branches demonstrate posterior probabilities for BI and bootstrap supports for ML, respectively.



**Fig. 3.** Charts from the comparison of abundance of MOTUs (a) and number of OTUs (b), also shared OTUs (c) between sites.

MOTUs (E, K and L) between pasture and farmland (Fig. 3c).

For two-way ANOVA the dataset was normally distributed and the variance across groups are homogeneous. The results of two-way ANOVA for MOTUs diversity indicated significant effects of land uses and location on the total number of OTUs and total density (Table 2). Furthermore, according to our dataset, land uses (pasture and farmland) differed significantly each other ( $t$ -test;  $t = 9.47$ ,  $p = 0.007$ ). However, the difference between sites were not significant.

Investigation of  $\beta$ -diversity partitioning detected that the spatial turnover portion was the major fraction (turnover = 0.98; nestedness = 0.005;  $\beta$ -diversity for Jaccard dissimilarity index = 0.99). The results of the examination (turnover) with MOTUs in quadrats. The results of phylogenetic  $\beta$ -diversity showed turnover patterns as well (turnover = 0.64; nestedness = 0.002; total phylogenetic  $\beta$ -diversity = 0.64). The summary of phylogenetic  $\beta$ -diversity results is shown by heat maps in Fig. 4.

### 3.3. Assemblage composition

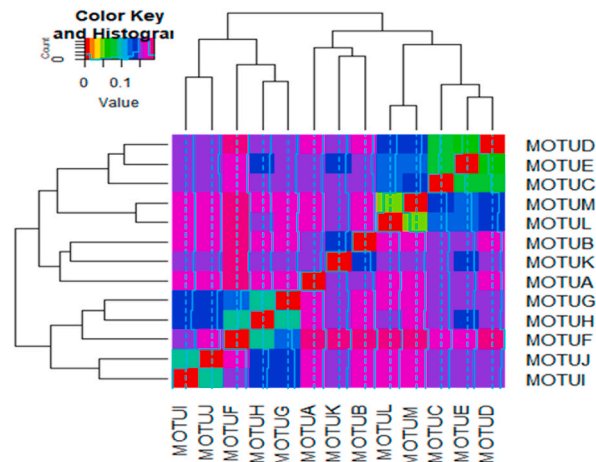
The PERMANOVA indicated significant effects of land uses on the MOTUs composition of earthworms (Table 2). SIMPER analysis indicated that the MOTUs (E, L, M, K, C, I) were the most responsible MOTUs for the observed significant difference between farmland and pasture habitats with a higher frequency of occurrence in the latter in the study area.

### 3.4. Relationship between earthworms and environmental variables

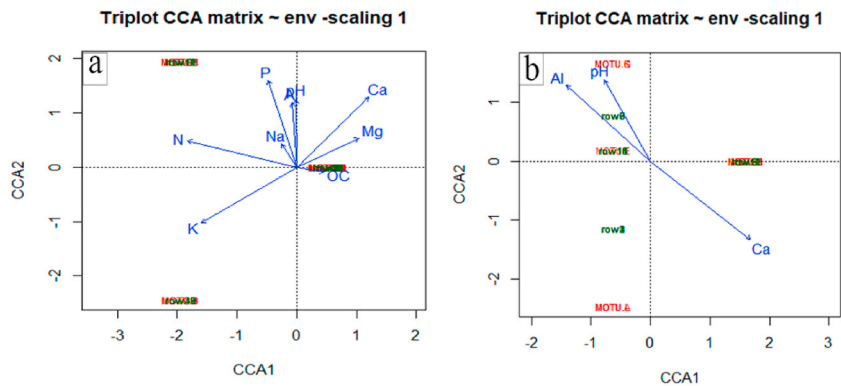
All measurement of soil physico-chemical properties and soil texture are shown in Table A.3. In the study area, the selected variables explained 10.52 of the total variation in MOTUs composition. Overall, X variables ("Constraints") explain a total of 4.902 bits of inertia, which is about 46 %, leaving about 53 % unexplained (Fig. 5a). In agriculture areas, there is a total inertia 5.25 and X variables explain a total of 1.656 bits of inertia, which is about 31 %, leaving about 68 % unexplained (Fig. 5b).

**Table 2**  
Summary of statistical analysis of ANOVA and PERMANOVA.

	ANOVA	PERMANOVA
Land use	$F_{1,40} = 8.001, p = 0.007$	$F_{1,31} = 5.608, p = 0.001$
Site	$F_{2,40} = 4.301, p = 0.0008$	$F_{2,31} = 3.113, p = 0.001$
Land use * Site	$F_{2,40} = 4.712, p = 0.0002$	$F_{2,31} = 3.425, p = 0.001$



**Fig. 4.** Phylogenetic Beta diversity heat map to compare the MOTUs of community differences between land uses. Color blocks represent distance values. The redder the color is, the closer the samples are, the higher the similarity is, and the bluer the distance is. In the heat map, the distance between samples can be seen through the clustering tree.



**Fig. 5.** CCA ordination diagram with MOTUs (triangles) and environmental factors (arrows). The ordination for study area (land use)(a) and just for farmland (crops) (b) The relative size of each arrow indicates the importance of that variable along the direction, pointing.

#### 4. Discussion

Our investigations have identified several MOTUs in the study area. We then compared the earthworm communities in farmlands and pastures and found that land use type significantly affects earthworm community abundance and composition.

##### 4.1. Identifying earthworm MOTUs

Identifying different species of earthworms poses several challenges due to their high level of phenotypic and morphological variability. Additionally, diagnostic characteristics may be lacking in juvenile stages, and the absence of expert identification services further complicates the process of identifying earthworm species [39]. Various studies have suggested that the COI-based identification system is a potent tool to identify earthworms with reliable evidence [83–86]. In this study, based on the results of phylogenetic trees, genetic distances, and species delimitations, we identified 13 MOTUs from six genera through barcoding studies. Identification of these earthworms based on morphological features was challenging. Barcoding provided more accurate results, but for most of the MOTUs, we could not determine the valid species names (Table 1). Assigning taxonomic names to a query sequence requires a DNA reference library with taxonomically pre-identified vouchers. In the case of Iranian species, there is not a complete genetic dataset available for defining them. However, several unique genetic datasets obtained from studies can aid in their identification. The low sequence coverage in databases also presents a challenge for identifying Oligochaete specimens in other studies [42].

The majority of the species identified in the study had been previously recorded in Iran, except for *Esenia nordenskioldi* (OUT J). *Esenia nordenskioldi* is known as a polymorphic and the most cold-tolerant earthworm that is distributed in Northern Eurasia widely [87]. Overall, composting earthworms of the genus *Eisenia* have an important role in soil [88], used for monitoring and bio-remediation of antibiotic-polluted soil [89]. The species just found from one site of pasture. OUT E (*Aporrectodea caliginosa*) and

OUT K (*Aporrectodea trapezoids*) are shared between farmland and pasture sites. In addition, OUT E is mostly seen in all sites (Fig. 3c). *Aporrectodea caliginosa* is a widespread species that has distributed in all temperate zones [90,91]. It is highly adaptable and ecologically resilient in agroecosystems. Previous research indicates that it is also abundant in Iran [47]. *Aporrectodea trapezoids* originated in the Palearctic realm but is now found throughout the world. In global soil management, *A. trapezoids* are a key species because they can adapt to extreme environmental conditions [92].

According to the results obtained from this research, all the genera found in agricultural lands belong to the Lumbricidae family. According to Lee [93], the Lumbridae family, particularly the genera *Lumbricus*, *Aporrectodea*, and *Eisenia*, possess the ability to adapt rapidly to new environments and exhibit high dispersion power. As a result, they could potentially establish themselves in new areas like farms and displace native species.

#### 4.2. How land use changes earthworm communities?

Our investigation, as shown by the ANOVA results, has revealed that not only the total number of OTUs in earthworms has changed between pastures and farmlands, but also the difference among sites has been observed to be significant. Furthermore, the composition of OTUs in earthworms has shown a significant difference between the two types of land use, as indicated by the PERMANOVA results. The number of MOTUs were higher generally for farmland earthworms (except one site; see Fig. 3), however in most cases, earthworms are most abundant in pasture sites. It is in line with results of Sing et al. [94]. They showed that grassland had a significantly higher abundance of earthworms than agricultural fields. Also, Evans and Guild [95] found that old pasture land at Rothamsted, England, was cultivated for six months, and then declined in earthworm numbers. Fragoso et al. [96] showed crops generally affect the abundance and diversity of communities; pastures are known to enhance earthworm biomass but may decrease taxonomic richness. Earthworm abundance patterns are therefore driven by changing land use patterns and shifting plant composition over time. It is also possible that pesticides and chemical fertilizers are causing a decrease in earthworm abundances in agricultural fields [97]. In general, earthworm community composition can be used to measure soil quality rather than earthworm numbers from an ecological perspective [98,99]. Pelosi and Römcke [100] with the goal of evaluating the link between a group of oligochaetes and agricultural practices indicated that oligochaete's abundance and composition are hugely impacted and interestingly, they can be used as good indicators of agricultural practices.

Our study found that there was turnover in earthworm communities between farmland and pasture land use, as evidenced by differences in  $\beta$ -diversity and phylogenetic  $\beta$ -diversity. Demetrio et al. [101] mentioned that high values of  $\beta$ -diversity and species turnovers between different habitat types could be a sign of having distinctive soil invertebrate communities, with many new species, especially the number of new ecosystem engineers, in each of these habitats.

#### 4.3. The effect of environmental variables on earthworm communities

A variety of environmental and edaphic factors can affect earthworms community and convert them when land use changes [101, 102]. The CCA findings (Fig. 5) demonstrated that the composition of MOTUs and distribution of earthworms between land uses (pasture and farmland) was noticeably influenced by multiple environmental variables. However, in agricultural systems (Fig. 5b), the correlation of MOTUs with calcium, Al and pH were higher than among other environmental factors. The Ca-content is probably due to the presence of an active calcareous gland in earthworms that actively secretes calcium carbonate-rich mucus into the esophagus [103]. This leads to the removal of excess Ca ions through casting activity and greatly increases the availability of calcium in the soil.

In the agricultural system, the effect of tillage on the earthworm population and biomass is greater than on the availability of food [104–106]. Different species behave differently in tillage practices and cropping systems; the larger species, *Aporrectodea* of MOTUs (C, D, H, and K) require surface substrate and live in permanent burrows, removed by frequent soil disturbance; While the endogenous and smaller MOTU species (E), benefit more from plowed crop residues [105,106]. In this research, the species of the Lumbricidae family are dominant (see Table 1) in the agricultural system and have endogenous life habits. Considering the rising *Aporrectodea* rate of land use change worldwide, especially in Iran, it is crucial to determine the impact of disturbance on earthworms.

Conversion of grassland to cropland has reduced earthworm population and affected soil organic matter [107]. The magnitude of the effects of earthworm invasion on nutrient cycling depends on the species composition of the invading earthworms as well as the history of land use in the agricultural system [108]. Agricultural systems have high levels of input compared to pastures. Chemical fertilizer has a negative impact on earthworms by increasing plant yield, which leads to more plant residues in fields after harvest, affecting earthworm mortality and distribution. In our research, we have observed the changing diversity and abundance of earthworms within the agricultural ecosystem (Fig. 3). We believe that it happens because conventional agriculture with the use of chemical fertilizers has caused the earthworm community to change towards endogenic, which has increased the processes of mineralization and humification. It has led to significant changes in the earthworm community composition [109]. However, when organic fertilizers and plant residues are introduced into the agricultural system, have observed a positive impact on earthworm fauna [110]. Among the arable lands that we studied, the covered land with clover, less disturbance, and inputs, has a higher frequency but less diversity (Fig. 3). Overall, reduced disturbance can positively influence the abundance of earthworms [4,111].

## 5. Conclusion

In this study, we recognized 13 MOTUs of earthworms from the Northern Zagros Mountains by molecular approaches. The comparison of farmlands and pastures in the study area revealed that there was a significant difference in diversity and density of



earthworms between land uses and sites. In addition, the results showed that the composition of earthworm communities has a significant difference. Furthermore, we found that farmlands have greater earthworm diversity, while pastures have mostly abundant earthworms. Species of earthworms behave differently in agricultural practices and cultivation systems due to the type of fertilizer, tillage, and complex interactions with microorganisms. In our study, the diversity of endogenous species and smaller OTU (E), family Lumbricidae, was dominant in the agricultural system. In cultivation systems, chemical inputs and organic materials from plant residues, cover crops, manure, or organic fertilizers determined the population of invasive species of earthworms. In our research, clover cultivation as a cover crop was more similar to pasture use. Considering the rising rate of land use change worldwide, especially in Iran, it is crucial to determine the impact of disturbance on earthworms. Therefore, such an assessment would definitely help to provide an action plan for the conservation of nature. Also, to address the limitation of genetic libraries, we propose more research on recognizing diversity of earthworms using barcoding methods throughout the world.

#### Data availability statement

The study's dataset was submitted to a publicly accessible repository. The genetic sequences associated with the study were deposited in the NCBI GenBank, and the accession numbers can be found in [Table 1](#) of the article. Data included in article/[Table 1](#)/referenced in article.

#### Ethics statement

This study was conducted with permission from the Ethics Committee of Shahid Beheshti University with the code of ethics 300/99.135758.

#### CRediT authorship contribution statement

**Shadi Karimifard:** Writing - original draft, Methodology, Investigation, Formal analysis, Data curation. **Reihaneh Saberi-Pirooz:** Writing - review & editing, Writing - original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Faraham Ahmadzadeh:** Writing - review & editing, Writing - original draft, Supervision, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Fatemeh Aghamir:** Writing - review & editing, Writing - original draft, Supervision, Project administration, Methodology, Conceptualization.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Appendices.

**Table A.1**  
Uncorrected genetic *p*-distances within earthworms using COI

	MOTU A	MOTU B	MOTU C	MOTU D	MOTU E	MOTU F	MOTU G	MOTU H	MOTU I	MOTU J	MOTU K	MOTU L	MOTU M
MOTU A													
MOTU B	0.152												
MOTU C	0.158	0.152											
MOTU D	0.139	0.178	0.072										
MOTU E	0.125	0.145	0.086	0.059									
MOTU F	0.192	0.189	0.182	0.196	0.182								
MOTU G	0.186	0.166	0.160	0.153	0.133	0.113							
MOTU H	0.152	0.152	0.139	0.165	0.165	0.096	0.100						
MOTU I	0.158	0.172	0.145	0.132	0.145	0.156	0.126	0.112					
MOTU J	0.158	0.158	0.145	0.165	0.158	0.149	0.160	0.152	0.099				
MOTU K	0.156	0.143	0.156	0.156	0.130	0.170	0.171	0.170	0.163	0.143			
MOTU L	0.172	0.172	0.125	0.145	0.139	0.156	0.166	0.145	0.158	0.172	0.136		
MOTU M	0.166	0.173	0.113	0.140	0.140	0.177	0.174	0.140	0.160	0.173	0.158	0.047	

**Table A. 2**  
Diversity of earthworms OTUs across six pasture and four farmland sites

Land Information	Land_use	MOTU A	MOTU B	MOTU C	MOTU D	MOTU E	MOTU F	MOTU G	MOTU H	MOTU I	MOTU J	MOTU K	MOTU L	MOTU M
Wheat	Farmland	1	0	0	0	1	0	0	0	0	0	0	1	0
Sugar Beet	Farmland	0	0	0	0	3	0	1	0	0	0	1	0	0
Alfalfa	Farmland	0	0	2	0	0	0	0	1	2	0	0	0	0
Clover	Farmland	0	0	0	0	5	0	0	0	0	0	0	0	0
Village Vennaie	Pasture	0	0	0	0	0	0	0	0	0	0	0	2	3
Village Koolidar	Pasture	0	0	0	0	0	0	0	0	0	0	2	0	0
Village Doran Dareh	Pasture	0	0	0	0	1	2	0	0	0	0	0	0	1
Village Zeresheh	Pasture	0	0	0	1	0	0	0	0	0	0	0	0	1
Village Gondal Gilan	Pasture	0	0	0	0	2	0	0	0	0	0	0	2	0
Village Zavarian	Pasture	0	1	0	0	0	0	0	0	0	1	0	0	0

**Table A.3**  
The soil chemical and physical properties (pH, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Al<sup>3+</sup>, K<sup>+</sup>, P, total N, and total C) and Soil texture (the percentage of Clay, Silt, and Sand) across land uses (farmlands and pastures)

Land Information	Al (ppm)	Ca (ppm)	K (ppm)	Mg (ppm)	Na (ppm)	P (ppm)	OC %	N %	Sand %	Silt %	Clay %	pH
Farmland- Wheat	65586 (13117.2)	92404 (18480.8)	19164 (3832.8)	13577 (2715.4)	8308 (1661.6)	1021 (204.2)	0.8 (0.16)	0.14 (0.028)	19.6 (3.92)	40 (8)	40.4 (8.08)	8 (1.6)
Farmland- Sugar Beet	76792 (15358.4)	67051	19456	16178	13074	894	1.27	0.14	17.6	46	36.4	8.2
Farmland- Alfalfa	67240 (13448)	92779	19382	12171	8687	1010	1.32	0.18	33.6	28	38.4	8.1
Farmland- Clover	80992 (16198.4)	65721	20648	16320	12894	804	1.34	0.14	17.6	46	36.4	8.3
Pasture-Village Vennaie	34326 (6865.2)	102412 (20482.4)	15334 (3066.8)	14287 (2857.4)	3952 (790.4)	609 (121.8)	1.23 (0.246)	0.09 (0.018)	18.6 (3.72)	34 (6.8)	47.4 (9.48)	7.8 (1.52)
Pasture-Village Koolidar	28668 (5733.6)	135140	11521	9888	2353	575	1.99	0.16	24.6	28	47.4	7.6
Pasture-Village Doran Dareh	37371 (7474.2)	85251 (17050.2)	13054 (2610.8)	11884 (2376.8)	2871 (574.2)	504 (100.8)	1.4 (0.28)	0.11 (0.022)	16.6 (3.32)	32 (6.4)	51.4 (10.28)	7.8 (1.56)
Pasture-Village Zeresheh	25625 (5125)	164798 (32959.6)	10110 (2022)	11221 (2244.2)	2769 (553.8)	449 (89.8)	2.5 (0.5)	0.11 (0.022)	20.6 (4.12)	32 (6.4)	47.4 (9.48)	7.7 (1.54)
Pasture-Village Gondal Gilan	26292 (5258.4)	87740 (17548)	18839 (3767.8)	12588 (2517.6)	5664 (1132.8)	544 (108.8)	1.66 (0.332)	0.14 (0.028)	28.6 (5.72)	36 (7.2)	35.4 (7.08)	7.6 (1.52)
Pasture-Village Zavarian	25683 (5136.6)	18035 (3607)	26846 (5369.2)	10363 (2072.6)	6084 (1216.8)	466 (93.2)	1.38 (0.276)	0.16 (0.032)	44.6 (8.92)	28 (5.6)	27.4 (5.48)	7.6 (1.52)

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