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Structure and diversity of earthworm communities in long-term irrigated soils with raw effluent and treated wastewater

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ABSTRACT

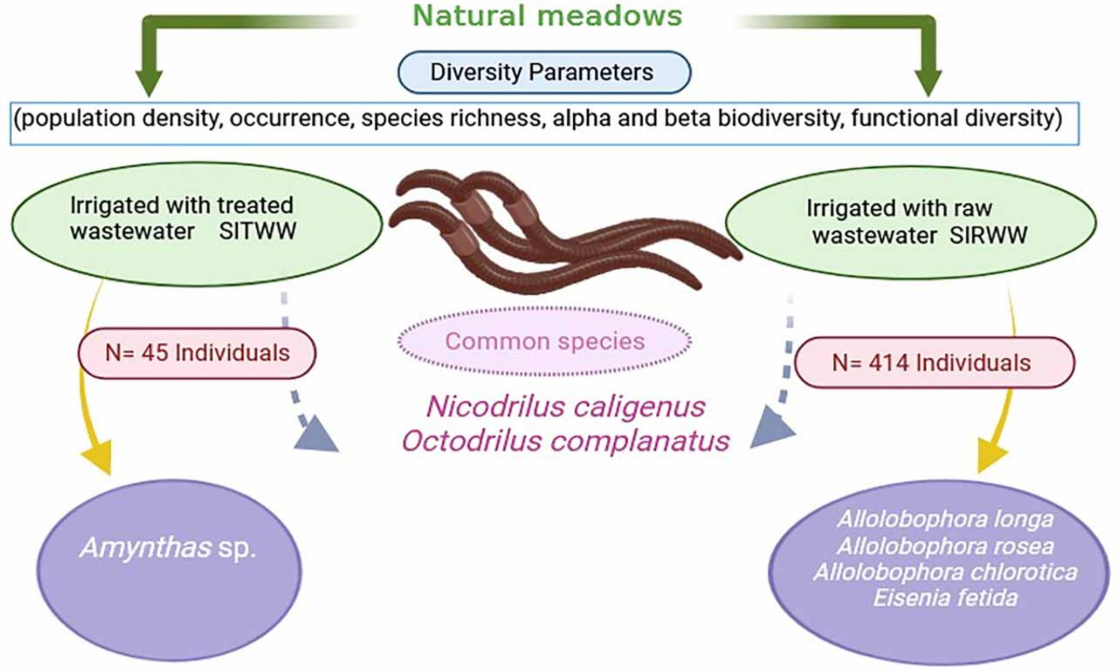
This study was conducted in two natural meadows: first, soils were irrigated with raw wastewater (SIRWW) and in the second, soils were irrigated with treated wastewater (SITWW). Earthworms were sampled in eight soil blocks spaced 10 m apart at each site. Earthworm community was characterized and compared using density, biomass, composition, structure, species richness, and diversity parameters. At both meadows, 459 earthworm individuals from two families and seven species were collected. The highest earthworm density and species richness were recorded at SIRWW. *Nicodrilus caligenus* was the most abundant species. Most of earthworm community parameters decreased significantly at SITWW. Only two species (*N. caligenus* and *Octodrilus complanatus*) were common between the two grasslands. Among the seven species identified at both meadows, four (*Allolobophora longa, Eisenia foetida, Allolobophora rosea, Allolobophora chlorotica*) were exclusively present in SIRWW, whereas a single species (*Amynthas* sp.) was characterized in SITWW. Three ecological earthworm groups (epigeic, endogeic, and anectic) were represented in SIRWW, with the dominance of endogeics. Further studies are needed to quantify pollution in this soils and the accumulation of pollutant load in earthworms. It is also important to highlight the relationship between the abundance and diversity of earthworms in these two ecosystems with soil biological activity.

Key words: biodiversity, earthworm community, natural meadows, soil bioindicators, vermifiltration, wastewater irrigation

HIGHLIGHTS

- Diversity and composition of earthworm community were studied in meadows receiving wastewater.
- Species richness and abundance varied between sites irrigated with raw and treated wastewaters.
- Grassland meadows irrigated with raw wastewater created a favorable environment for earthworms.
- Endogeic species tolerate large variations of environmental conditions in wastewater-irrigated lands.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Soils are complex ecosystems that sustain diverse organisms, including bacteria and pedofauna, which play an important role in maintaining their functions (Byrne 2022; Ding & Eldridge 2022). Macrofauna has a significant influence on the entire soil fauna community and, therefore, on ecosystem functions (Ohgushi *et al.* 2018). Among the soil fauna, the earthworms, invertebrates belonging to the phylum Annelida, are ecosystem engineers capable of modifying the soil environment they inhabit (Sizmur & Richardson 2020; Bora *et al.* 2021). Their incredible architectural heel contributes to the modification of the soil structure. This biological reworking of soil is accomplished through the movement of materials known as bioturbation. Thus, earthworms play a key beneficial role in soil structure, function, and productivity (Liu *et al.* 2019). Dhiman & Pant (2022) consider them as indicators of soil biological activity. They can withstand high concentrations of soil pollutants and play a vital role in their effective removal (Zeb *et al.* 2020). Earthworms have also shown considerable potential for the remediation of soils polluted by heavy metals such as Pb, Cd, and Ni (Ahadi *et al.* 2020) and organic contaminants including polychlorinated biphenyls, polybrominated diphenyl ethers, polycyclic aromatic hydrocarbons, and pesticides (Zeb *et al.* 2020). The decontamination mechanisms include the accumulation of high concentration of heavy metals in the bodies of earthworms, and/or absorption of the water soluble and extractable fractions of heavy metals by direct contact with earth skin or by gut absorption after ingestion of contaminated organic matter.

Earthworms drive multiple soil processes, but their specific impact on soil functions differs among species and ecological categories (Arrázola-Vásquez *et al.* 2022). They play an important role in organic matter decomposition and nutrient recycling (Coleman 2013; Singh *et al.* 2019; Yang *et al.* 2020; Cheng *et al.* 2021). This gives them a critical role in terrestrial ecosystems as an important biomarker for toxicology research (Bart *et al.* 2018; Chen *et al.* 2020; Zhou *et al.* 2020; Dhiman & Pant 2022; Edwards & Arancon 2022). Depending on the source of the contaminants, earthworms can be used to rehabilitate contaminated soils (Al-Maliki *et al.* 2021). They are relatively tolerant of toxic elements in soils (Fründ *et al.* 2011; Richardson *et al.* 2020). Sizmur & Richardson (2020) found that both endogeic and epigeic earthworms increase the mobility of potentially toxic elements in soil and in casts, and all ecological groups (epigeic, endogeic, and anecic) mobilize potentially toxic elements during soil passage through the earthworm gut. Through the vermifiltration process, earthworms are used to treat organic pollutants in wastewater (Kanaujia *et al.* 2020; Arora *et al.* 2021, 2022) as well as chemical and pathogenic contaminants (Mohan *et al.* 2022) and even better and more stable performance has been found in the removal of antibiotics from hospital wastewater (Shokouhi *et al.* 2020). To survive in such an environment, earthworms have developed effective immune defense mechanisms against invading microorganisms existing in wastewater (Romo *et al.* 2016). Furthermore, earthworms play a vital role in shaping and maintaining healthy soil ecosystems, making them valuable indicators of soil pollution and the effects of receiving wastewaters (Ahadi *et al.* 2020). Earthworms are a diverse group of soil-dwelling organisms that contribute to soil fertility and structure through their feeding and burrowing activities. They consume organic matter, such as dead plant material, and excrete nutrient-rich casts, which enhance soil nutrient cycling and increase its water-holding capacity. As such, earthworms are considered ecosystem engineers, influencing soil physical, chemical, and biological properties (Liu *et al.* 2019; Sizmur & Richardson 2020; Arrázola-Vásquez *et al.* 2022). In the context of soil pollution, earthworms act as sensitive bioindicators. Their abundance, diversity, and behavior can be significantly affected by pollutants present in the soil (Ouahrani 2003; Sekhara-Baha 2008). Various studies have demonstrated the adverse effects of contaminants, such as heavy metals, pesticides, and organic pollutants, on earthworm populations (Nahmani *et al.* 2003; Zeb *et al.* 2020). Changes in earthworm community composition and reduced population densities can indicate soil degradation and the presence of pollutants (Dlamini & Haynes 2004; Singh *et al.* 2021; Ahmed *et al.* 2022). Moreover, the physiological responses of earthworms to pollutants, such as alterations in enzyme activities or reproductive patterns, provide additional insights into the severity of soil contamination (Pelosi *et al.* 2014; Romo *et al.* 2016; Chen *et al.* 2020; Cheng *et al.* 2021).

The use of reclaimed wastewater for irrigation is increasingly recognized as a way to reduce water consumption by promoting the reuse of treated wastewater (Boudjabi *et al.* 2023). The drawback is that reclaimed wastewater can be a vector of soil contamination, and its use is expected to alter soil properties, especially the microbial community (Guedes *et al.* 2022). In recent years, the potential of using earthworms to treat sewage sludge, domestic wastewater, and human feces is increasing (Arora *et al.* 2020). Sinha *et al.* (2008) suggest that the bodies of earthworms behave as biofilters and they remove contaminants from wastewater at a rate of 80–90%. According to Zeb *et al.* (2020), earthworms can withstand high concentrations of soil pollutants and play a key role in removing them effectively.

From the point of view of diversity, it has been reported by European Commission (2016) that more than 7,000 earthworm species have been described worldwide, but the expected number of species is much higher. Earthworm abundance tends to decline during the dry or extremely cold season and reaches the highest densities and biomass when climatic and soil conditions are more favorable (Singh *et al.* 2019). The diversity of the earthworm community in a given environment is influenced by the characteristics of climate, soil, vegetation, and organic resources, as well as the history of land use and soil disturbance (Edwards & Arancon 2022). Based on the overall strategy of biodiversity conservation and ecosystem services provided by organisms, it is of current and future interest to assess the functional and structural biodiversity of arable soils (van Capelle *et al.* 2012). Biological diversity has been suggested to be linked to ecosystem functioning (Scherer-Lorenzen *et al.* 2022). It is in this context that earthworms are regularly used as bioindicators for management changes or soil contamination (Fründ *et al.* 2011; Pelosi *et al.* 2014).

Wastewater is a common source of soil pollution, as it often contains various contaminants that can adversely affect soil quality (Ababsa *et al.*, 2020; Boudjabi *et al.* 2023). Wastewater, whether from domestic, industrial, or agricultural sources, may contain high levels of organic matter, nutrients, heavy metals, and harmful chemicals (Shokouhi *et al.* 2020). When wastewater is discharged onto land or used for irrigation, it can significantly impact soil ecosystems (Chenchouni *et al.* 2022; Guedes *et al.* 2022). In this context, this study assumes that earthworms can act as reliable indicators of the effects of wastewater on soil health. Accordingly, monitoring earthworm populations and assessing their responses to wastewater application can provide valuable information about the potential risks and impacts of this practice. By studying earthworm communities in soil receiving wastewaters, this study aimed at gaining insights into the pollution levels and the overall health of the soil, facilitating the development of effective soil management strategies and pollution mitigation measures. This paper investigates the effect of irrigation with treated and raw wastewater on the abundance and diversity of earthworms in natural meadow grasslands. In fact, we have evaluated the impact of the reuse of these two ‘non-conventional waters’ on the biological component of the soil characterized by earthworms, and we hypothesize that the soil irrigated with treated water will be more abundant in earthworms than the soil irrigated with raw sewage. The site irrigated with raw wastewater (SIRWW) can be considered as a natural bio-filter that uses the biological component of the soil as a decontamination agent that protects the soil against the polluting components of raw wastewater.

2. MATERIALS AND METHODS

2.1. Study area

The two sites that were the subject of this study are located in the province '*wilaya*' of Setif, situated in the high plains of eastern Algeria. The studied sites are irrigated meadow grasslands. The first site is located at the peri-urban area of the city of Setif (36°11′3.66″N, 05°22′50.66″E, elevation = 984 m a.s.l., https://goo.gl/maps/4qLUePJR61LFsFWA7). It is a large area of natural meadow grassland that has been irrigated for a long time with raw wastewater discharged from the city of Setif. The soil of this meadow is sub-permanently submerged by water (i.e. gley soil). The second site is another grassland located 1 km east of Beni Fouda city at Setif (36°17′98.00″N, 05°36′56.00″E, elevation = 833 m a.s.l., https://goo.gl/maps/6WWZ7evjKtY8EPCL9). The irrigation of this meadow was ensured by treated wastewater generated from the city's wastewater plant. The irrigation of the second meadow has been practiced since the installation of the wastewater treatment plant in 2006.

Landscapes of the region of Setif include mainly plains used for rainfed cereal cropping and mountains occupied by Mediterranean sclerophyllous forests. The climate is Mediterranean semi-arid with hot and dry summers (maximum temperature of July = 32.5 °C) and cold winters (minimum temperature of January = 0.6 °C). Precipitation is low and erratic, ranging from 228.1 to 503.8 mm/year (Ababsa *et al.* 2020). Soil characteristics of the two meadows are represented in Table 1.

2.2. Earthworm sampling and counting

Sampling was conducted in April 2021, when earthworm activity was at its maximum. We used the hand-sorting standard sampling method, which consists of hand-sorting all earthworms included in a block of soil 50 cm square and 30 cm deep. Separation of earthworms from the soil matrix was performed onsite. For each site, i.e. meadow soils irrigated with raw wastewater (SIRWW) and meadow soils irrigated with treated wastewater (SITWW), earthworms were sampled in eight soil blocks spaced 10 m apart. The sampling points were chosen along the diagonal in each meadow. All collected earthworms were placed in plastic jars (300 mL) containing ethanol, and upon arrival at the laboratory, the earthworms were rinsed and dried on paper, after which they were weighed and counted. Then the specimens were preserved in 70% ethanol before being identified. The identification of earthworm species was based on morphological characteristics that involves carefully observing and analyzing various physical traits and features of the earthworm's body, including: the external morphology such as color, size and shape of the body, the number of segments (annuli), and any distinctive markings or structures on the skin; segmentation and clitellum (its location, shape, and coloration); arrangement, number, and distribution of setae on each segment; and shape and structure of the earthworm's head, as well as its mouthparts. Some features might require microscopic examination. This could involve looking at structures like the prostomium, mouthparts, and reproductive organs in finer detail. To accurately identify a species, the observed characteristics are compared to established taxonomic keys, identification guides, and existing literature on the available species descriptions of the region (Bouché 1972; Omodeo *et al.* 2003; Sekhara-Baha 2008). The identified earthworm species were classified into three ecological groups: epigeic (litter dwelling), endogeic (shallow burrowing), and anecic (deep burrowing) (Bouché 1972).

Table 1 | Soil characteristics of the two meadow sites: SIRWW and SITWW

Soil properties	SITWW	SIRWW
pH	8.0	7.5
Electrical conductivity (mS/cm)	0.592	1.231
P_2O_5 (ppm)	181.7	253.2
Total nitrogen (%)	0.26	0.26
Organic matter (%)	3.78	7.52
Total $CaCO_3$ (%)	33.88	17.94
Soil moisture (%)	20	90
Soil texture	Clayey	Clayey

2.3. Data analysis

The assessment of earthworm biodiversity was determined using several descriptors based on the absolute abundance (n_i) measured for each earthworm species at each sample in the two meadows. The relative abundance (RA) was determined as the ratio of the number of individuals of a species (n_i) to the total number of individuals (N) of all species ($RA = n_i/N \times 100$). Frequency of occurrence (Occ) was calculated for each species as the ratio of the number of samples where the species was present (m_i) to the total number of samples (M) realized ($Occ = m_i/M \times 100$). Based on occurrence values, species were classified as: very accidental species (VA) when Occ < 10%, accidental species (AC) when Occ = [10–25%], common species (CM) when Occ = [25–50%], and constant species (CN) when Occ ≥ 50%.

All earthworm individuals of all species pooled were weighed to determine total fresh biomass (B) per sample. Species richness (S) was defined as the total number of species present at a given sample or a batch of samples (meadow). At the sample level, abundance-to-species richness ratio (N/S) and biomass-to-species richness ratio (B/S) were computed to standardize abundances and biomasses to species richness.

Earthworm alpha diversity was explored using Shannon diversity index (H), with $H = -\Sigma((n_i/N) \times \log_2(n_i/N))$; Pielou's evenness index (E), $E = H/\log_2(S)$; Simpson reciprocal index (SRI) diversity, where $SRI = (N(N-1))/\Sigma(n_i(n_i-1))$; and SRI/S ratio. The latter ratio and E vary from 0 (indicating uneven distribution of species densities within the community, often related to the dominance of 1–2 species) to 1 (indicating high balance between densities of populations). The range of SRI values included between 1 (indicating low diversity) and S (indicating high diversity) (Chenchouni 2017).

Using the software EstimateS version 9.1 (Colwell 2013), species richness was estimated and interpolated in each meadow separately and in both meadows combined. In each case, estimates of S was carried out using four asymptotic species richness estimators: first-(S_{Jack1})-order and second-(S_{Jack2})-order Jackknife estimators, Chao1 (S_{Chao1}), and Chao2 (S_{Chao1}) estimators. We employed sample-based rarefaction curves to compare species richness in sampled meadows, and accumulation curves for species richness interpolations to a sample size of 100, which represented a sampling effort higher by 12.5 times than the reference sample size in each meadow ($M = 8$).

2.4. Statistical analysis

The R software (version 4.2.1) was used for statistical analyzes of the data (R Core Team 2022). Sample-based data of earthworm species and community were summarized using basic statistics (mean, standard deviation, minimum, maximum, and coefficient of variation) for each sampling meadow. Community parameters for SIRWW and SITWW were plotted using the R package {ggplot2}. Prior testing the variation of earthworm community parameters, i.e. those related to density, biomass, composition, structure, species richness, and diversity; Shapiro-Wilk test was used for verifying normality. Then differences of these parameters among SIRWW and SITWW were analyzed using Kruskal–Wallis rank sum tests. The variation of each species abundance per sample between SIRWW and SITWW was also tested using the same procedure. The interrelationships between earthworm community parameters were analyzed Pearson correlation tests separately for each sampled meadow. The obtained correlation matrices were mapped in an interactive plot using the R package {corrplot}. The statistical significance of the applied tests was set at the threshold ($p < 0.05$).

3. RESULTS

3.1. Community composition and systematics

The results including all earthworm species collected at the two sites studied (SIRWW and SITWW) are reported in Table 2. For both meadows sampled, a total of 459 individuals of earthworms were collected, of which 90.2% were collected from SIRWW. The three ecological groups: epigeic, endogeic, and anecic were represented, with a dominance of endogiec earthworms. Only seven species, attached to two families, were identified based on all earthworm individuals collected (Table 2).

The meadow with SIRWW enclosed the highest earthworm abundance, with a total of 414 individuals classified into six species, five genera and one family (Lumbricidae). *Nicodrilus caligenus* was the most abundant species (62.32% of the total abundance), whereas *Octodrilus complanatus* represented 18.12% of individuals, and the remaining four species had abundances less than 10%, namely *Allolobophora chlorotica* (RA = 9%), *Allolobophora longa* (RA = 4.59%), *Eisenia fetida* (RA = 3.14%), and *Allolobophora rosea* (RA = 2.17%). With only 45 individuals (RA = 9.2% of the total), the abundance of earthworms collected in SITWW was much lower compared to SIRWW. The community included three species belonging to three genera and two families (Lumbricidae and Megascolecidae), of which *O. complanatus* was the most abundant (RA = 95.6%) and the other two species (*N. caligenus* and *Amynthas* sp.) represented each 2.2% of the total abundance

Table 2 | Systematic list, ecological groups, absolute abundance (n_i and N), relative abundance (RA, %) and occurrence (occ, %) of earthworm species collected in the two meadow soils irrigated with raw wastewater (SIRWW) and treated wastewater (SITWW)

			SITWW					SIRWW					Overall				
Family	**Species**	**Ecological groups**	**ni ± SD**	**N**	**RA**	**Occ**	**Scale**	**ni ± SD**	**N**	**RA**	**Occ**	**Scale**	**ni ± SD**	**N**	**RA**	**Occ**	**Scale**
Lumbricidae	*Octodrilus complanatus* (Dugés, 1828)	En–An	5.4 ± 8.9	43	95.6	50.0	CN	9.4 ± 15.7	75.0	18.1	50.0	CN	7.4 ± 12.5	118	25.7	50.0	CN
Lumbricidae	*Nicodrilus caligenus* (Bouché, (1972)	En	0.1 ± 0.4	1	2.2	12.5	AC	32.3 ± 38.6	258.0	62.3	75.0	CN	16.2 ± 31.2	259	56.4	43.8	CM
Lumbricidae	*Allolobophora longa* (Ude, 1885)	An	–	–	–	–	–	2.4 ± 4.8	19.0	4.6	25	CM	1.2 ± 3.5	19	4.1	12.5	AC
Lumbricidae	*Allolobophora rosea* (Bouché, (1972)	En	–	–	–	–	–	1.1 ± 2.2	9.0	2.2	25	CM	0.6 ± 1.6	9	2.0	12.5	AC
Lumbricidae	*Allolobophora chlorotica* (Savigny, 1826)	En	–	–	–	–	–	5.0 ± 14.1	40.0	9.7	12.5	AC	2.5 ± 10.0	40	8.7	6.3	VA
Lumbricidae	*Eisenia fetida* (Savigny, 1826)	Ep	–	–	–	–	–	1.6 ± 4.6	13.0	3.1	12.5	AC	0.8 ± 3.3	13	2.8	6.3	VA
Megascolecidae	*Amynthas* sp.	–	0.1 ± 0.4	1	2.2	12.5	AC	–	–	–	–	–	0.1 ± 0.3	1	0.2	6.3	VA
Total			5.6 ± 8.7	45	100			51.8 ± 33.3	414	100			28.7 ± 33.5	459	100		

Species abundances are given in mean ± standard deviation (n_i ± SD) the total at all samples (N). Ecological groups (Ep: epigeic, En: endogeic, En–An: endogeic–anecic, An: anecic). Scales of occurrence (CN: constant species, CM: common species, AC: accidental species, VA: very accidental species).

(Table 2). The Kruskal–Wallis rank sum test showed that only *N. caligenus* species were site-dependent ($\chi^2 = 7.08$, $p = 0.008$). While for the other species, no statistically significant difference ($p > 0.05$) was recorded between the two meadows.

According to specific values of species occurrence frequency (Occ) at soil samples collected at SIRWW (Table 2), *E. fetida* and *A. chlorotica* were accidental and rare (Occ $\leq$ 12.5%), *A. longa* and *A. rosea* were considered common species (12.5 < Occ < 50) and *O. complanatus* and *N. caligenus* were constant species (Occ > 50%). At SITWW, *O. complanatus* was classified as a constant species as it occurred in 50% of samples, while *N. caligenus* and *Amynthas* sp. occurred as accidental species.

3.2. Density, biomass and Alfa diversity estimates of earthworms

Figure 1 summarizes the values of earthworm community parameters (density, biomass, composition, structure, species richness, and diversity) obtained for the two meadows. The boxplots highlighted a large variability in data between and within SIRWW and SITWW. The SIRWW was more abundant and richer in earthworms with an N/S ratio of 34 $\pm$ 31.52. On average it had 51.75 $\pm$ 33.28 individuals and 2 $\pm$ 0.76 species, while SITWW showed an abundance of 5.63 $\pm$ 8.73 individuals and 0.75 $\pm$ 0.71 species. The edaphic conditions of the meadow with SIRWW allowed the development of earthworm biomass 12 times more important than the biomass recorded in SITWW (22.23 $\pm$ 17.6 g vs. 2.73 $\pm$ 5.73 g, respectively). The biomass-to-species richness ratio was also higher at SIRWW. Earthworm diversity and community structure were characterized using Shannon index, Pielou's evenness index, Simpson reciprocal index, and SRI/S ratio, which denoted the highest scores in SIRWW with 0.8 $\pm$ 0.59, 0.66 $\pm$ 0.44, 1.8 $\pm$ 0.7, and 0.91 $\pm$ 0.13, respectively (Figure 1); compared to the low values recorded in SITWW (0.13 $\pm$ 0.35, 0.13 $\pm$ 0.35, 0.75 $\pm$ 0.71 and 0.63 $\pm$ 0.52, respectively).

3.3. Variations of density, biomass and diversity parameters

The results of the Kruskal–Wallis rank sum test (Figure 1) revealed significant differences ($K^2 > 3.87$, $p < 0.05$) among the two studied meadows in almost all the parameters characterizing the community of earthworms. These significant variations concerned earthworm abundance (number of individuals), species richness, abundance-to-richness ratio, biomass, biomass-to-richness ratio, Shannon diversity index, evenness, and SRI. Only values of SRI/S ratio did not show a significant difference ($K^2 = 0.11$, $p = 0.741$) between SIRWW and SITWW according to this test.

3.4. Interrelationships between diversity parameters

Out of the 36 correlation tests realized between the studied parameters in each meadow (Figure 2), 22 were significant ($p < 0.05$) in SIRWW and 14 in SITWW. Overall and for both meadows, non-significant correlations ($p > 0.05$) were recorded between the pairs *N*–(*S*, *H*, *E*, SRI, SRI/*S*), *B*–(*S*, *E*), and SRI/*S*–(*N/S*, *B*, *B/S*, *H*, and *E*). The significant correlations observed in *N* (with N/*S*, *B*, and *B/S*) were all positive, these four parameters were all positively correlated. The parameters of density and biomass (*N*, *N/S*, *B*, and *B/S*) were negatively correlated with diversity and structure indices (*H*, *E*, and SRI); however, these negative correlations were significant only in SIRWW. Species richness and community diversity parameters, i.e. *S*, *H*, *E*, and SRI, were all significantly and positively auto-correlated. The majority of negative correlations among the studied parameters was observed in SIRWW, in which S revealed significant correlations with all parameters except *B* and SRI/S, and N/S with all parameters except SRI/S. The significant correlations observed with SRI/S were established with S ($p = 0.004$) and SRI ($p = 0.004$).

3.5. Traits of earthworm ecological groups

Data on abundance, biomass, species richness, occurrence parameters of earthworm ecological groups recorded at the two studied meadows are reported in Table 3. In SITWW, the endogeic–anecic group was the most abundant (N = 43 individuals). Endogeics were marked by only one individual, while no earthworms belonging to the epigeic and anecic groups were collected. Moreover, all ecological groups were present in SIRWW, with a dominance of endogeics (307 individuals), followed by endogeic–anecic, anecic and epigeic with abundance values of 75, 19, and 13, respectively. The highest value of RA was recorded by the endogeic–anecic group (RA = 95.6%) in SITWW, while RA was higher in the endogeic group (RA = 74.2%) of SIRWW. The endogeic group was represented by four species which had highest species richness score in SIRWW, while the other groups, when present, were represented by only one species. The highest value of N/S ratio was recorded by the endogeic–anecic group in SITWW, whereas in SIRWW, the two groups endogeic–anecic and endogeic had almost similar N/S ratios, 75 and 76.75, respectively. Earthworms of the endogeic–anecic ecological group occurred regularly in both meadows (Occ = 50%), which assigned them to the class of constant groups. The same occurrence class was distinguished in SIRWW

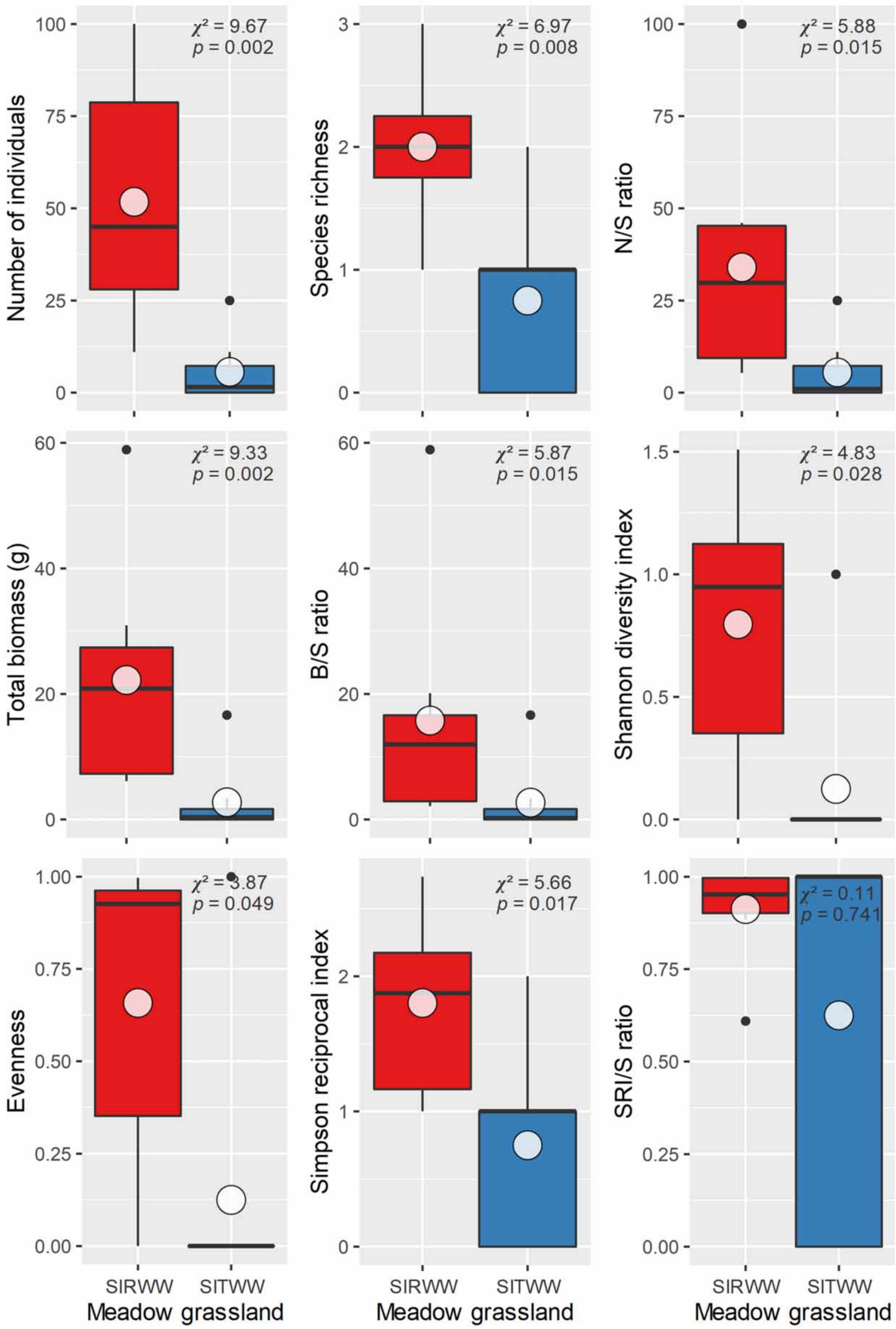


Figure 1 | Boxplots displaying statistics and values of earthworm density, biomass, composition, and diversity parameters in meadow soils irrigated with raw (SIRWW) and treated wastewater (SITWW). Chi-squared statistics (χ^2) and *p*-values are results of Kruskal–Wallis rank sum tests of differences among the two meadows.

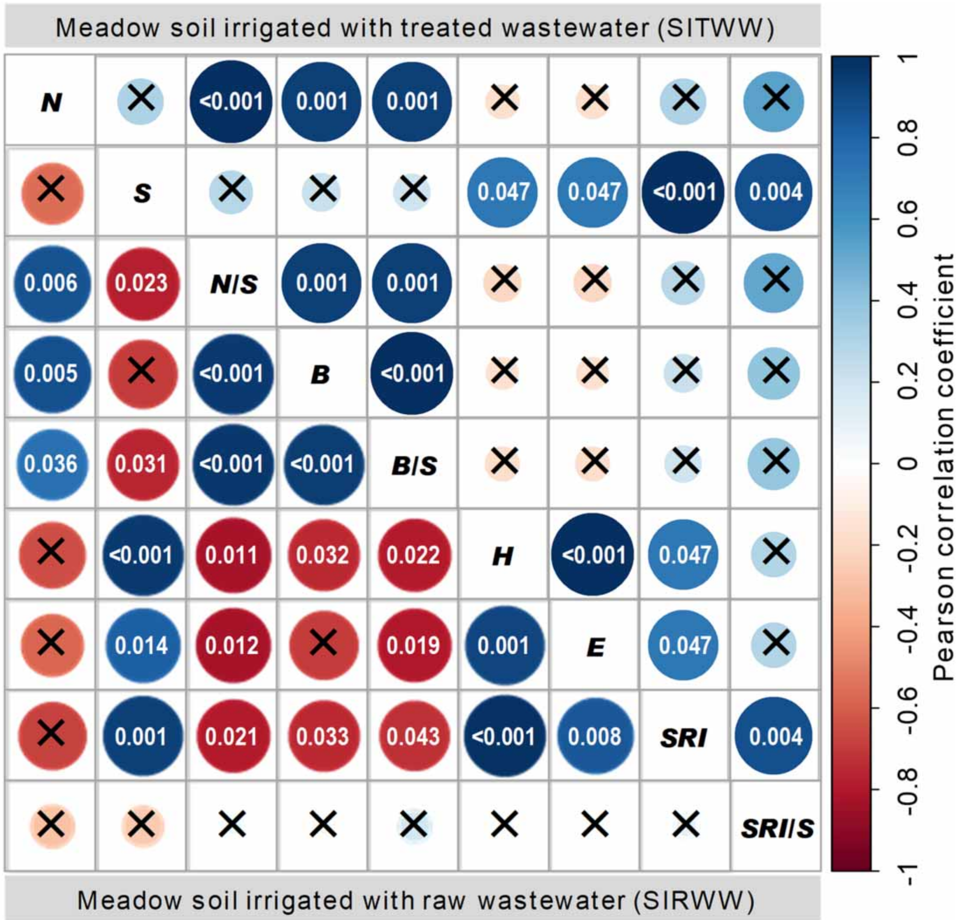


Figure 2 | Probability values of correlation coefficients of Pearson's correlation tests between density, biomass, and diversity parameters of earthworms collected in soils of the two meadows (SITWW above the diagonal and SIRWW below the diagonal). *P*-values are superimposed over the solid circles whose size and color are proportional to Pearson correlation coefficient scores. Correlations marked with a cross are non-significant ($p > 0.05$). (Variable abbreviations: *N*: abundance (number of individuals), *S*: species richness, *N/S:* abundance-to-species richness ratio, *B:* total biomass, *B/S:* biomass-to-species richness ratio, *H:* Shannon diversity index, *E*: Pielou's evenness index, SRI: Simpson reciprocal index, and SRI/S ratio.)

for the endogeic group (Occ = 87.5%), which was classified as incidental (Occ = 12.5%) in SITWW. For the latter meadow, epigeic and anecic groups occurred as accidental and common earthworms, respectively.

3.6. Species richness rarefication

The values of the four estimators of species richness applied in this study are summarized in Figure 3. The first-order Jackknife species richness estimator (S_{Jack1}) showed that rarified species richness was expected to increase by 34.8% in SITWW and 23.1% in SIRWW. For both meadows combined, estimated species richness was 9.8 species exceeding the observed species richness ($S = 7$), which indicated that the overall completeness of earthworm inventory was 72%. The application of the second-order Jackknife estimator (S_{Jack2}) revealed that species richness was 48.3% ($S_{\mathrm{Jack2}} = 5.8$ species) and 24.6% ($S_{\mathrm{Jack2}} = 7.96$) higher than the observed richness in SITWW and SIRWW, respectively. For the samples of two meadows pooled, estimated species richness was expected to stretch to 10.8 species ($S_{\mathrm{obs}} = 7$ species). Chao1 species richness estimator demonstrated that inventory completeness was 100% in SIRWW and in the two meadows combined ($S_{\mathrm{Chao1}} = S_{\mathrm{obs}}$). In SITWW, inventory completeness was estimated to 75.4% as S_{Chao1} was 3.98 species and $S_{\mathrm{obs}} = 3$ species. Furthermore, the application of the Chao2 estimator revealed slight to moderate increases in species richness, with $S_{\mathrm{Chao2}} = 3.8$, $S_{\mathrm{Chao2}} = 6.29$ and $S_{\mathrm{Chao2}} = 9.11$, which matched up inventory completeness levels of 78.9, 95.4, and 76.8% in SITWW, SIRWW, and both meadows combined, respectively (Figure 3).

Table 3 | Number of individuals, relative abundance, species richness, *N/S* ratio, and occurrence frequency for the ecological groups of earthworms collected in meadow soils irrigated with raw wastewater (SIRWW) and treated wastewater (SITWW) in northeastern Algeria

	Ecological groups				
Sampled meadows	**Epigeic**	**Endogeic**	**Endogeic–Anecic**	**Anecic**	**Not determined**
Total density 'N'					
SITWW	–	1	43	–	1
SIRWW	13	307	75	19	–
Relative abundance (%)					
SITWW	–	2.2	95.6	–	2.2
SIRWW	3.1	74.2	18.1	4.6	–
Species richness '*S*'					
SITWW	–	1	1	–	1
SIRWW	1	4	1	1	–
N/S ratio					
SITWW	–	1	43	–	1
SIRWW	13	76.75	75	19	–
Occurrence frequency (%)					
SITWW	–	12.5	50	–	12.5
SIRWW	12.5	87.5	50	25	–

3.7. Species richness interpolation

Overall, the rarefaction curves increased with the increase in number of samples and then reached a plateau (Figure 4). The interpolation curves of species richness showed that S_{est} appeared to reach stability from the 12th sample in SITWW and from the 20th sample in SIRWW. The predicted values of S_{est} (mean $\pm$ SD [lower 95%–upper 95% of confidential intervals]) at 100 samples were 3.8 $\pm$ 1.38 species (CI: 1.09–6.51), 6.87 $\pm$ 1.64 species (CI: 3.66–10.09) and 9.11 $\pm$ 3.15 species (CI: 2.93–15.28) for SITWW, SIRWW, and both meadows combined, respectively. These projected species richness scores were

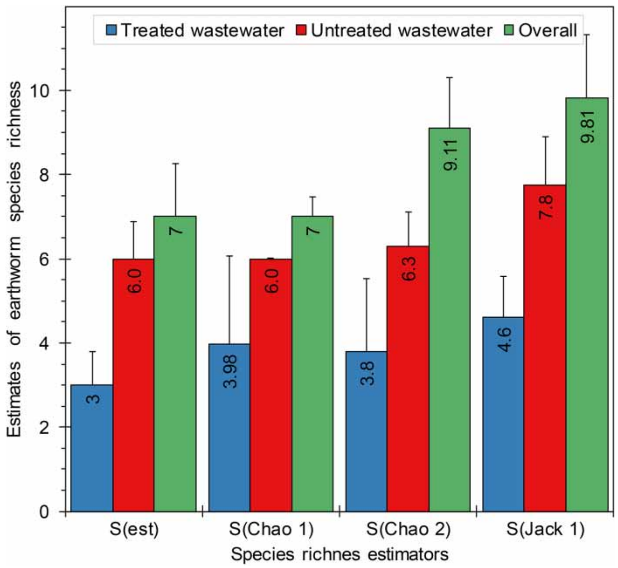


Figure 3 | Observed (S_{est} = analytical) and estimated (S_{Jack1}, S_{Jack2}, S_{Chao1}, and S_{Chao2}) earthworm species richness recorded at meadows irrigated with treated and untreated wastewater, and for both meadows combined (Overall). Rectangular bars and error bars represent mean values and standard deviations, respectively, of species richness estimates averaged over 100 randomizations.

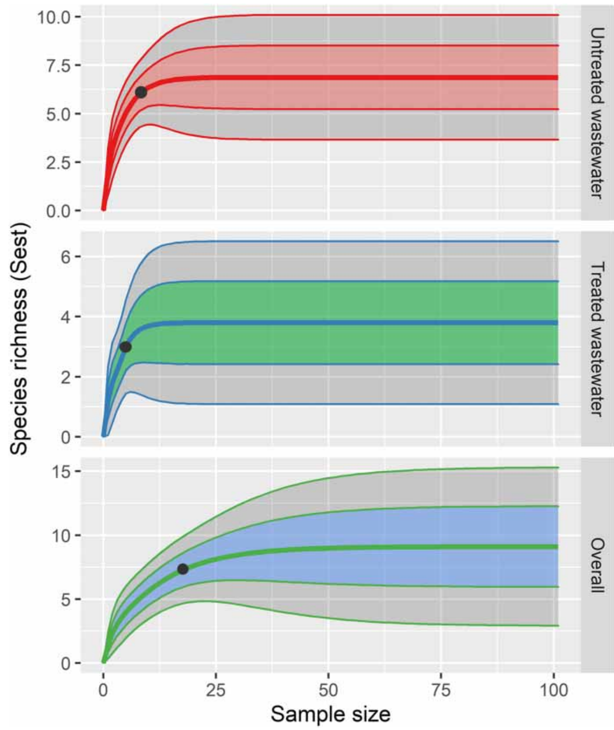


Figure 4 | Sample-based interpolation curves of species richness estimated for earthworms at meadows irrigated with treated and untreated wastewater, and for both meadows combined (overall). Light grey-shaded areas represent lower and upper bounds of 95% confidence intervals for $S_{(est)}$. Color-shaded areas indicate ± standard deviations of $S_{(est)}$ determined over 100 randomizations. The black solid circle refers to the reference sample size used in rarefication.

associated to specific densities of 900; 5,175; and 2,869 individuals, respectively. Accordingly, with the increase if number of samples to 100, the number of earthworm species was expected to increase by 26.6, 14.5, and 30.14% for SITWW, SIRWW, and both meadows combined.

3.8. Analysis of similarity (beta diversity)

The Venn diagram indicated that only two species (*N. caligenus* and *O. complanatus*) were common between the two meadows (Figure 5). Out of the seven species identified in this study, four were exclusively present for SIRWW (*A. longa*, *E. fetida*, *A. rosea*, *A. chlorotica*). In contrast, SITWW was characterized by the exclusivity of a single species (*Amynthas* sp.). The application of qualitative similarity indices (Jaccard and Sørensen) showed that two meadows had a low similarity of 28.6 and 44.4%, respectively. Similarly, quantitative similarity estimated using Morisita-Horn index and Bray–Curtis index indicated low resemblance values with 28.8 and 20.8%, respectively. However, both raw and estimated metrics of either Chao-Jaccard and Chao-Sørensen showed high similarity scores with 86.4, 97.8, 92.7, and 98.9%, respectively (Figure 5). These high scores were related to the abundance-based coverage estimations (ACE) of species richness in SITWW (ACE $\approx$ 4 species), which was greater than the observed species richness (three species).

4. DISCUSSION

This study analyzed and compared various parameters (density, biomass, composition, structure, species richness, and diversity) of the earthworm community living in long-term irrigated soils with wastewater in meadow grasslands in a semiarid region of Algeria (North Africa). In this study, we collected a total of 459 individuals classified into two families (Lumbricidae and Megascolecidae), five genera and seven species, with the family Lumbricidae being the most represented with 458 individuals and six species. According to Edwards & Arancon (2022), these two families are the most ecologically important in Europe, North America, Australia, and Asia. Mainly endemic in the Palearctic region, including Europe and the north region of North Africa, the family Lumbricidae is spread all over the world, mainly by humans. Earthworms naturally disperse through various

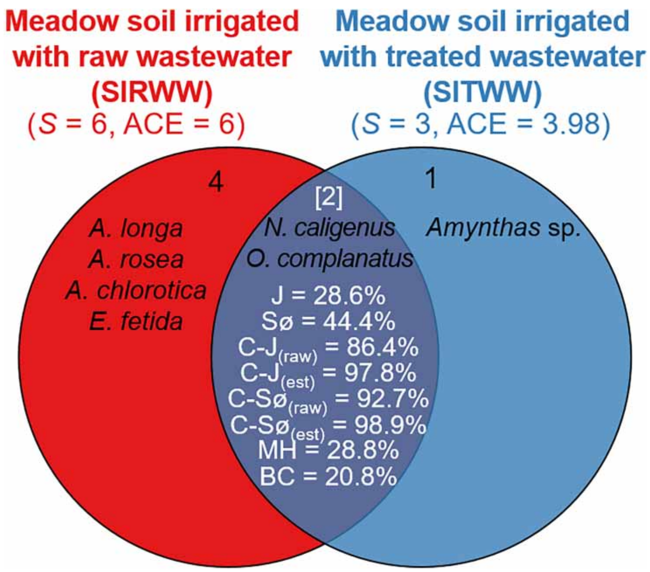


Figure 5 | Venn diagram displaying observed (*S*) and estimated (ACE: abundance-based coverage estimation) species richness of earthworms recorded in meadow soils irrigated with raw wastewater (SIRWW) and treated wastewater (SITWW) in northeastern Algeria. Figures in black are number of exclusive species of each meadow, whereas the white number between square brackets represents the number of shared species among these meadows. Binomials of earthworm species are given in italics, with only initials of genera. Similarity statistics (in %) are represented within the overlapped area of the diagram (*J*: the classic Jaccard index, Sø: the classic Sørensen index, $C–J_{(raw)}$: raw Chao's abundance-based Jaccard index, $C–J_{(est)}$: estimated Chao's abundance-based Jaccard index, $C–Sø_{(raw)}$: raw Chao's abundance-based Sørensen index, $C–Sø_{(est)}$: estimated Chao's abundance-based Sørensen index, MH: Morisita–Horn index, BC: Bray–Curtis index).

mechanisms. One way is through natural events like heavy rainfall, where earthworms can be carried in streams or run-off. Additionally, accidental transportation by humans, known as anthropochore transport, can occur when earthworms are unintentionally carried along with plants or other materials. Earthworm cocoons can also be transported by birds and other animals (zoochory), clinging to their feet or bodies. The accidental introduction of earthworms into soil where plants grow can significantly impact their dispersal patterns. Moreover, passive dispersal can happen through activities such as tractor usage, where earthworms can be inadvertently spread, influencing the rate of population expansion in newly colonized agricultural fields (Mathieu *et al.* 2018). According to Reynolds (2018), a total of 35 species belonging to six families were identified in Algeria, including: Acanthodrilidae: 3 species, Criodrilidae: 2 species, Glossoscolecidae: 1 species, Haplotaxidae: 1 species, Hormogastridae: 1 species, and Lumbricidae: 27 species. Earthworms are found worldwide in soils with sufficient moisture to support and sustain them, except in arid lands such as deserts. Their abundance is extremely variable, ranging from only a few individuals to more than 2,000 per m² (Bora *et al.* 2021; Edwards & Arancon 2022). They are common worldwide in forests and natural grasslands as well as in agrosystems (Philips *et al.* 2019; Zerrouki *et al.* 2022).

In total, we recorded the presence of seven earthworm species in both irrigated meadow, with a specific richness of six species in SIRWW and only three species in SITWW. Of the 35 species identified in Algeria, Bazri *et al.* (2013), determined 18 species in eastern Algeria at 62 sites distributed over different bioclimatic zones; whereas Sekhara-Baha (2008), recorded 11 species in the Mitidja plain; and Ouahrani (2003), determined 11 species in the Constantinois region. In meadows irrigated with raw treated and agricultural effluents, Ababsa *et al.* (2020), determined three species at Setif. Following the study on the effects of grassland management on earthworm communities under ambient and future climatic conditions (Singh *et al.* 2021), earthworm communities were significantly impacted by different grassland types, with earthworm abundance and biomass decreased in grasslands with high intensification use and low plant diversity.

The distribution of earthworm densities per studied meadow revealed that SIRWW was more abundant ($N = 414$ individuals) in earthworms than SITWW ($N = 45$ individuals). According to Ababsa *et al.* (2020), the irrigation with raw urban wastewater, despite its richness in organic and particulate matter, resulted in increased earthworm density, higher soil porosity and water transfer.

Environmental conditions directly affect the abundance and diversity of earthworms (Ahmed *et al.* 2022; Ding & Eldridge 2022). In the present study, despite the commonality between the two meadows, as an irrigated grassland ecosystem, it appears that SIRWW created favorable conditions for the development of earthworm populations. Earthworms can withstand high concentrations of soil pollutants and play a vital role in their effective removal. They can remove contaminants from the soil or help degrade non-recyclable chemicals; these are the basis for vermifiltration, which has proven to be an alternative and inexpensive technology to treat contaminated soils (Zeb *et al.* 2020).

In SIRWW, earthworms are an integrated component of the pedofauna that participates in the remediation of pollution, especially in meadows and grasslands, which cover about 40% of global inland surface and harbor the greatest number of earthworms (Edwards & Bohlen 1996). These ecosystems have high economic, ecological and biodiversity values due to their role in providing fodder for livestock and retaining high levels of carbon in the soil (Lenhart *et al.* 2015). The ecological conditions at SIRWW allowed for the development of a high abundance and richness of earthworms, with an earthworm biomass 12 times greater than the biomass at SITWW. The reason for this high abundance and richness could be attributed to the adaptation of earthworms to the quality of the irrigation wastewater. Rodriguez-Campos *et al.* (2014), reported that the presence, and in some cases the high abundance, of earthworms in contaminated environments suggests their high eco-physiological tolerance and ecological plasticity.

Apart from soil moisture that was considered in this study, and which is dependent on air and soil temperature as well as other physicochemical soil properties, soil factors are reported to influence the abundance and diversity for earthworms. According to Mishra *et al.* (2020) and Zerrouki *et al.* (2022), soil temperature, moisture, and other edaphic factors are key regulators of earthworm abundance and activity in nature. Earthworms are most sensitive to the hydraulic properties of the soil, and in times of drought, they move to deeper soil layers for protection (Johnston *et al.* 2014). The studies of Philips *et al.* (2019), Singh *et al.* (2019), and Ahmed *et al.* (2022), reported the presence of a significant positive relationship between earthworm density with soil moisture and rainfall. Thakur *et al.* (2018) reported that soil moisture is a major factor determining soil biological activity, while Al-Maliki *et al.* (2021) concluded that earthworms are more sensitive to changes in soil temperature than to the effects of moisture content and thus can be used as a bio-indicator of soil quality. It is true that earthworm activity depends on adequate soil moisture availability, but not all species have the same moisture requirements, and within a species, the moisture requirements of earthworm populations in different parts of the world can be very different. For instance, *A. chlorotica*, *A. longa*, and *A. caliginosa* are among the earthworm species that can survive long periods submerged in water (Edwards & Arancon 2022).

The soil of the studied meadow with the highest earthworm abundance was submerged in raw wastewater (gley soil), while the grassland with the lowest earthworm abundance was characterized by low soil moisture compared to SIRWW. Furthermore, a high-performance of treatment against chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP), total suspended solids and even heavy metals has been approved by the vermifiltration of domestic and industrial wastewater (Namaldi & Azgin 2023). Removal rates were 82.1, 92.8, 96.4, and 96.3%, respectively, for COD, NH_4^+-N, NT and TP as reported by Das & Paul (2023). Studies reported the presence of significant positive relationships between earthworms and the content of soil organic carbon, where high soil organic carbon levels favored high earthworm abundance (Dlamini & Haynes 2004; Johnston *et al.* 2014; Ahmed *et al.* 2022).

The ecological categories of earthworms help us understand the associated ecological processes and establish mechanistic links between earthworm community structure and ecosystem function (Hsu *et al.* 2023). Despite the dominance of endogeics (four species), all three ecological groups are represented in this study (Table 2). The results of the study conducted by Singh *et al.* (2021) reveal the dominance of endogeic species in terms of total abundance and biomass. Our findings are in agreement with previous studies, where endogeic species dominance in grasslands is clearly described (Didden 2001; Butt *et al.* 2022). This may be related to the fact that endogeic species tolerate large variations in environmental conditions. Similar findings were reported by Mantoani *et al.* (2022), where a dominance of the endogenic earthworms (*A. chlorotica*, *A. caliginosa,* and *A. rosea*) was observed in all sampled plots across five grassland sites.

Some earthworm species are widely distributed and are not characteristic of any particular site/habitat/ecosystem. In our study, the two species, *O. complanatus* and *N. caligenus* were ubiquitous regardless of the quality of irrigation water. Nahmani *et al.* (2003) reported that *A. caliginosa* was dominant in unpolluted grasslands (density = 45 ind./m²). This species consumes high proportions of soil organic matter and microorganisms in its diet (Potapov *et al.* 2019). Fonte *et al.* (2009) reported that it is a cosmopolitan species and commonly found in temperate agroecosystems worldwide. A large-bodied anecic worm, *O. complanatus* is present from North Africa and Spain through southern European countries and Cyprus to Turkey and the Levant region (Pavlíček & Csuzdi 2016). It is typically restricted to wet meadows (Chenchouni 2017), and common in grasslands and pastures, with a wide distribution in Europe and North Africa (Monroy *et al.* 2007). Because

A. caliginosa is sensitive to metal toxicity and has high ecological plasticity and adaptability in agroecosystems, it is considered an excellent bioindicator model of pollution and environmental status (Bouché 1972; Otmani *et al.* 2018). Adults of this endogeic species were dominant in all agricultural systems sampled by Lemtiri *et al.* (2018). In our study, *A. chlorotica* was present only at SIRWW (in gley soil) with an AR of 8.7%. According to Plum & Filser (2005), this species was dominant I gley soil of wet grasslands. It is a species known for its tolerance to wet soils (Zorn *et al.* 2008). *A. chlorotica* and *A. rosea* species are probably the most widespread earthworm species in the world (Szederjesi 2017). The taxon *Amynthas* sp. exhibited a more restricted distribution where we collected only a single individual from SITWW. *Eisenia fetida* worms are widely used to test the toxicity of pollutants (Jiang *et al.* 2020; Chenchouni *et al.* 2022). Weight change and cocoon production of this earthworm as well as mortality were significantly affected when salinity increases (Owojori *et al.* 2009; Yang *et al.* 2022). Raiesi *et al.* (2020) reported that salinity increased lead (Pb) toxicity to the life cycle and activity of *E. fetida*. In our study, this species was recorded only at SIRWW where soil electrical conductivity is twice higher than of SITWW.

5. CONCLUSION AND RECOMMENDATION

This study explored the diversity of earthworms in the rhizosphere of two meadow grassland ecosystems, SIRWW and SITWW. Our results revealed a good abundance and specific richness, as well as a higher biomass of earthworms in SIRWW compared to SITWW. Given the high abundance of *N. caligenus*, we suggest to set up an experimental study aiming at using this species in a vermifiltration system for urban wastewater treatment. Thus, we suggest carrying out other studies on the chemical quality of soils and quantifying the accumulation of pollutant load in the body of earthworms. Therefore, it is important to study the biological activity of soils under irrigation with both types of water to consider the relationship of abundance and biodiversity of earthworms with microorganisms, soil respiration, as well as the enzymatic activity of the soils.

The findings of this study hold significant relevance for regions beyond its immediate context. By investigating earthworm communities under contrasting wastewater irrigation practices, the study provides insights applicable to diverse global settings facing similar challenges. As wastewater utilization for irrigation gains prominence worldwide, understanding its impact on soil ecosystems is crucial. The observed higher earthworm abundance, species richness, and biomass in SIRWW highlight the potential benefits of such practices. The suggestion to explore the utilization of abundant, well-adapted and resilient species in vermifiltration systems for urban wastewater treatment introduces an innovative solution with broader implications. Furthermore, the call for studies on soil chemical quality, pollutant accumulation in earthworms, and the interplay between earthworms, microorganisms, soil respiration, and enzymatic activity underscores the need for comprehensive assessments of soil health. These findings resonate across regions grappling with sustainable agricultural practices, environmental conservation, and efficient wastewater management, making this study's insights invaluable for shaping informed decision-making globally.

AUTHORS' CONTRIBUTIONS

N.A. was involved in conceptualization, methodology, resources, investigation, writing – original draft, writing – review and editing. S.F. was involved in visualization, methodology, resources, investigation. H.C. was involved in formal analysis, visualization, writing – original draft, writing – review and editing. R.L. was involved in investigation. K.B. was involved in writing – review and editing. M.B. was involved in investigation. M.K. was involved in conceptualization and investigation. Please refer the CRediT taxonomy for the term explanation.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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