J. Hydrol. Hydromech., 73, 2025, 2, 190–199
https://doi.org/10.2478/johh-2025-0014

sciendo Journal of Hydrology and Hydromechanics Institute of Hydrology SAS

Introducing earhtworms to generate sustainable microenvironments able to modify soil hydrological properties on vineyards

José Carvajal-Pachón[1], Laura Cambronero-Ruiz[1,2], Francisco Serrano-Bernardo[3], Víctor Hugo Durán Zuazo[4], Jesús Rodrigo-Comino[1,2*]

[1] Departamento de Análisis Geográfico Regional y Geografía Física, Facultad de Filosofía y Letras, Campus Universitario de Cartuja, Universidad de Granada, 18071 Granada, Spain.
[2] Andalusian Research Institute in Data Science and Computational Intelligence, DaSCI, University of Granada, 18071, Granada, Spain.
[3] Departamento de Ingeniería Civil, ETSI Caminos, Canales y Puertos, Universidad de Granada, Campus Fuentenueva, s/n, 18071-Granada, Spain.
[4] Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA). IFAPA Center "Camino de Purchil", Granada, Spain.
[*] Corresponding author. E-mail: jesusrc@ugr.es

Abstract: Water infiltration and other physical soil properties are crucial determinants of soil quality and productivity. While it's established that earthworms can enhance soil structure, there's limited research quantifying their impact on these processes, particularly in vineyards. This study assesses the effect of introducing earthworms (*Lumbricus terrestris*) into soils under controlled conditions, focusing on improvements in soil infiltration, respiration, aggregate stability, moisture, and other key soil properties within Mediterranean vineyards. The research was conducted from December 2023 to April 2024, utilizing experimental microenvironments designed to mimic the natural conditions of vineyard soils in Granada, Spain. Results demonstrated that earthworm activity significantly increased both infiltration capacity and rate. Notably, infiltration was twice as fast in the treated plots from the third measurement onward. Furthermore, earthworms enhanced organic matter accumulation, with gains exceeding 1%, and boosted soil respiration by over 50 ppm. Although aggregate stability showed less dramatic improvement, the data indicate that earthworm-created galleries foster beneficial microenvironments for soil dynamics. These findings highlight the potential of earthworms as regenerative agents in degraded agricultural soils, emphasizing their capacity to support sustainable agricultural practices and mitigate the effects of climate change. Further long-term research in real-world settings is recommended to validate the scalability of these results.

Keywords: Earthworms; Viticulture; Soil hydrology; Climate change mitigation; Soil management.

1 INTRODUCTION

Soil is fundamentally composed of minerals and organic matter, and it harbours a diverse community of macro- and microorganisms of both animal and plant origin, sustained by air, water, and nutrients (Panagos et al., 2024). It is well-established that the proportions of these components vary considerably, influenced by factors such as parent material, climate, microbiota, topography, and the temporal scale of soil formation, as well as prevailing environmental conditions and anthropogenic activities (Lal et al., 2004). Globally, soil is regarded as a thin layer overlying the geosphere, developed over extended periods—ranging from decades to millennia—through the weathering of surface rocks by water, wind, and temperature fluctuations (Porta et al., 2003; FitzPatrick, 2013). Moreover, since the latter third of the 20th century, the ecological functions of soils, previously underappreciated, have gained recognition (Adhikari and Hartemink, 2016). These functions encompass biomass production, habitat provision, and serving as a source of raw materials, including food and fibers (Kibblewhite et al., 2007).

It is widely acknowledged that animals play a critical role in soil formation, conservation, and development (Hole, 1981). These organisms can be broadly classified into three primary groups (Swift et al., 1979). Firstly, macrofauna, including earthworms (*Lumbricus terrestris*) and insects such as flies and larvae, are responsible for transforming organic residues into organic matter (Siddiqui et al., 2024; Liao et al., 2024). Secondly, mesofauna, encompassing bacteria and fungi, are crucial for decomposing organic matter and releasing essential nutrients (Grinhut et al., 2007). Lastly, microfauna, which includes protozoa and nematodes, contribute to various soil processes (Edwards and Arancon, 2022). Beyond their biological roles in transforming and decomposing organic matter, these organisms also facilitate the creation of pores and galleries within the soil. These structural modifications are vital for aeration, water infiltration and storage, and promoting root growth (FAO, 2013).

Vineyard soils were selected for this study due to the abundance of existing data, enabling a more accurate evaluation of earthworm effects. Furthermore, as an experimental study, this soil type was chosen for its well-established knowledge base. To comprehensively assess soil quality and fertility, a suite of chemical, physical, and biological indicators, crucial for the proper functioning of the soil ecosystem, should be considered (Smith et al., 2023). Chemical indicators encompass elements that influence water quality, potential toxic elements, and essential nutrients or minerals (Bünemann et al., 2018). Given that crops, like most plants, absorb mineral nutrients from the soil through their roots, soil hydrology is fundamental for the sustainable management of agricultural systems. It is estimated that approximately 8,000 earthworm species exist, of which only about 30% have been studied and classified, with an even smaller proportion successfully domesticated (Guanche, 2015).

Earthworms constitute the largest animal biomass in many terrestrial temperate ecosystems (Domínguez et al., 2009), though their role in vineyard soils has been under increasing scrutiny at larger scales over the past decade (Paoletti et al., 2008). They perform two fundamental functions: humus production and soil tillage, thereby modifying soil structure and porosity. Previous studies have consistently demonstrated that earthworm activity improves soil structure through bioturbation, resulting in increased porosity, aggregation, and water infiltration (Kassam, et al., 2016). For many decades, their presence also influences nutrient cycling by accelerating organic matter decomposition and altering microbial communities, thereby enhancing soil fertility (Bhadauria and Saxena, 2010). Research across different ecosystems, including forests (Lal et al., 2004) grasslands (Eisenhauer and Scheu, 2008), and agricultural lands, (Barley, 1959) consistently supports these findings, indicating that the fundamental mechanisms of earthworm-soil interactions are consistent across various land use types. Despite the varied management practices in vineyards, such as tillage (or no-tillage) and the use of chemical or organic inputs, the overarching impact of earthworms on soil properties aligns with findings from other land use types. Sheep and goat grazing has a long-standing tradition in vineyards (Conrad et al., 2022). However, the increased use of chemical products and pesticides, the decline in grazing activity, and a lack of awareness regarding its benefits have led to a decrease in this practice in our country, despite its resurgence in viticultural regions of France (Moraine, 2024). Numerous projects aim to integrate animals into the management and improvement of cultivated soils (Minea et al., 2022). While limited, these initiatives represent an innovative approach with promising results, particularly concerning hydrological processes (Eldridge et al., 2022). However, none of these efforts to date directly utilize earthworms–natural soil tillers–within vineyards to enhance infiltration capacity, while simultaneously considering other associated properties such as organic matter content, aggregate stability, pH, and soil respiration facilitated by their gallery systems.

This study hypothesizes that earthworm activity will enhance soil infiltration and water storage capacity through the creation of extensive gallery systems. Furthermore, it is expected that earthworm introduction will positively influence specific physical and chemical soil properties. Building upon established principles, this research focuses on vineyard soils to determine whether specific soil conditions or management practices modulate earthworm-driven modifications under future field conditions. The primary objective of this study is to evaluate the impact of introducing *Lumbricus terrestris* into Mediterranean vineyard soils, aiming to improve key soil hydrological properties. Specifically, this study aims to: i) Analyze the effects of earthworm activity on soil water infiltration capacity and rate; and, ii) Evaluate changes in soil properties, including aggregate stability, organic matter content, and soil respiration, following earthworm introduction.

2 MATERIAL AND METHODS

2.1 Experimental setup

Two wooden boxes filled with soil have been designed as experimental plots under controlled laboratory conditions to simulate two different micro-environments. Soil was collected from two different depths: the first 10 cm of the soil profile (tilled horizon) and the 10–25 cm layer (vines´ roots development). The vineyard soil originates from Bodegas Calvente and was extracted according to its soil horizons, distinguishing between soil found at depths of 0–10 cm and 10–25 cm. The soil from each depth was placed in separate containers for transportation. This study focuses on a 10-year-old vineyard owned by Bodegas Calvente, located in the arid Mediterranean climate of Villamena, Lecrín Valley, Granada. The vineyard, stretching 1 km long, is situated on slopes of 10-15% and consists of calcareous Anthrosol (IUSS-WRB, 2022) developed on a geological base of marble, dolomite, and alluvium. The vineyard is cultivated using conventional methods, incorporating organic matter, NPK fertilizers, and fertigation through drip irrigation every 10 days during summer. The four red grape varieties—Cabernet Sauvignon, Tempranillo, Petit Verdot, and Merlot—are grown in a landscape transformed from original forests to agricultural land, primarily vineyards, olive, and almond orchards. The vineyard management reflects a balance between traditional practices with the use of fertilizers and chemicals and modern techniques such as the use of cover crops to ensure soil conservation and optimal productivity. In Figure 1, the location and the exact area where the soil was collected for the research are shown.

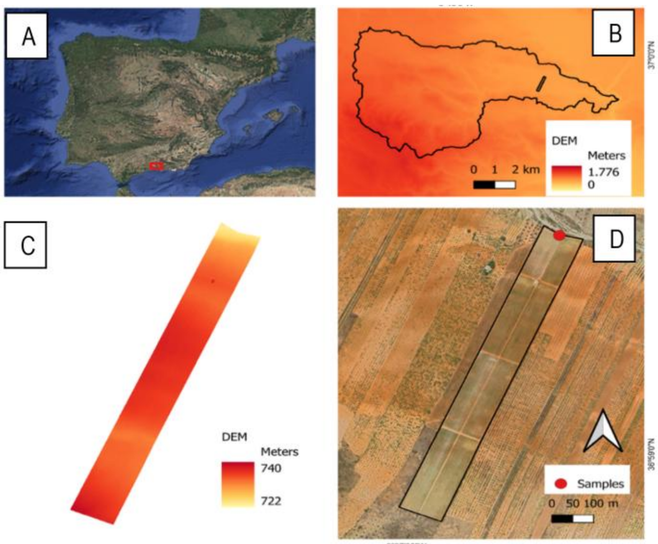


Fig. 1. Localisation of collected soils in vineyards. A. Localisation in the Iberian Peninsula; B: Localisation of the vineyard at the catchment scale; C: Digital elevation model showing topographical differences. D: Aerial photography showing location of sampled soils (PNOA, 2022, IGN).

2.2 Experimental design and monitoring

The earthworm species selected for this study was Lumbricus terrestris, an endogeic species native to Europe. This choice was based on three primary considerations: (i) its native status, ensuring no non-native species were introduced and facilitating replicability in other European countries; (ii) its relatively long lifespan of 4 to 8 years; and (iii) its availability, as it is commercially bred for reptile feed. The experimental plots were established outdoors at the Higher Technical School of Civil Engineering (Escuela Técnica Superior de Ingeniería de Caminos, Canales y Puertos), University of Granada. Two micro-environments were simulated using rectangular wooden boxes constructed from durable seven-layer laminated wood, each measuring 1 meter in length, 30 cm in width, and 30 cm in height, providing an internal volume of approximately 0.1 m^3. One box served as the treatment plot, into which 60 L. terrestris individuals were introduced. The other box remained undisturbed as a control, allowing for accurate comparison of data and realistic assessment of earthworm-induced changes.

To replicate the vineyard hillside conditions, the boxes were positioned at a 7.5º slope, as depicted in Figure 1. Soil was layered within the boxes to maintain the original soil profile, with the 10–25 cm layer placed first, followed by the top 10 cm layer.

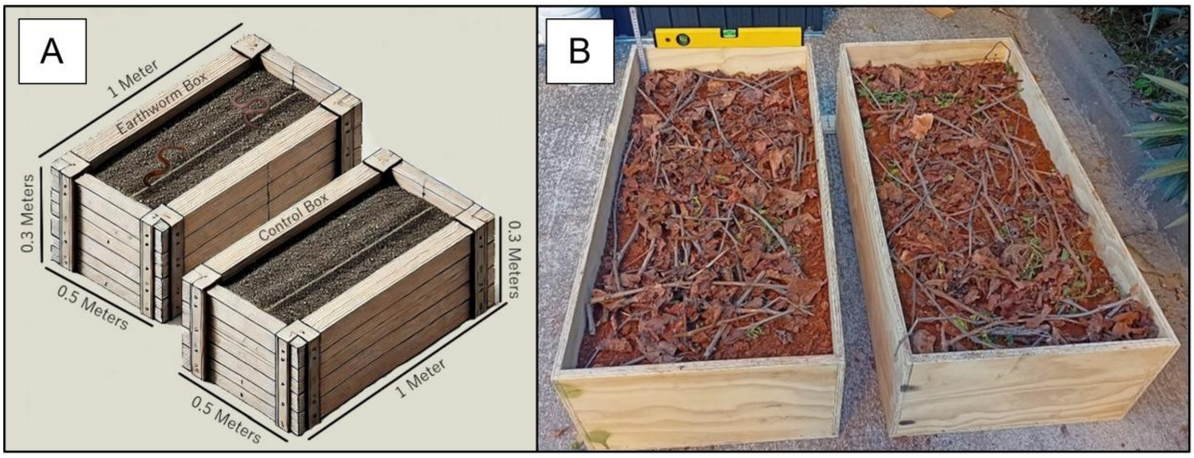


Fig. 2. Design of both experimental plots (A) simulating two micro-environments (with earthworms and control conditions) and situation after introducing the earthworms (B).

Table 1. Sampling plan and climatic data considering experiments, soil sampling and meteorological conditions.

Date	Precipita-tion (mm)	Tempera-ture Min (°C)	Tempera-ture Max (°C)	Soil Col-lection	Soil Depo-sition	Pre-Sample Testing	Introduction of Earthworns	Routine Sampling	Sampling Number
13/12/2023	–	–	–	X	–	–	–	–	–
18/12/2023	–	–	–	–	X	–	–	–	–
18/01/2024	36.8	9.9	17.3	–	–	X	X	–	1
19/01/2024	5.2	7.6	17.4	–	–	–	–	–	–
30/01/2024	0	-1.9	26.7	–	–	–	–	X	2
13/02/2024	54.2	2.6	22.2	–	–	–	–	X	3
29/02/2024	33.4	1.9	23.2	–	–	–	–	X	4
13/03/2024	55	0	20.2	–	–	–	–	X	5
01/04/2024	91.6	3.1	28.7	–	–	–	–	X	6

The experimental plots were positioned parallel to each other, separated by a 10 cm gap. To track changes within the plots, a soil sampling plan was implemented at 15-day intervals, as detailed in Table 1, which also includes key research dates alongside the routine sampling schedule. Routine soil sampling, including the pre-earthworm introduction sampling, was conducted at two locations within each box: one at the upper section and one at the lower section. At each location, soil samples were collected from both the surface layer (0–10 cm) and the deeper layer (10–25 cm). This resulted in a total of four soil samples per plot every 15 days. In addition to soil sampling, two experimental procedures were performed: (i) soil infiltration capacity was measured using a mini-disc infiltrometer (METER, model-S), and (ii) soil respiration was measured using a portable CO2 gas analyzer (EGM-5).

2.3 Soil properties associated to hydrological processes

Antecedent soil moisture was assessed prior to each sampling and experiment by drying soil samples at 60°C for a minimum of 24 hours. For the analysis of other variables, dried samples were sieved to a particle size of less than 2 mm. Aggregate stability was determined using a wet-sieving procedure. Prior to sieving, soil aggregates were carefully selected. These aggregates, representing compacted soil structures of varying sizes, were subjected to sequential wet sieving with 25 mL of distilled water, followed by 25 mL of a sodium hexametaphosphate solution (2 g/L). Aggregate stability was then calculated using the following formula (Eq. 1) (Amézketa, 1999; Fernández-Gálvez et al., 2012):

$$\%\, Aggregates = \frac{Soil\ 2nd}{(Soil\ 1st + Soil\ 2nd)} \quad (1)$$

Aggregate stability results were expressed as numerical values between 0 and 1, where values closer to 0 indicate higher soil aggregate stability, and values closer to 1 indicate lower stability. Soil pH was measured using a pH meter (PH50 VIOLAB DHS) with a 1:3 soil-to-water ratio. Soil organic matter content was determined using a muffle furnace. For each sample, three 2 g subsamples were placed in crucibles and heated at 430°C for 24 hours. The percentage of organic matter was calculated using the loss on ignition method, as described by Schulte and Hopkins (1996). Soil respiration, an indicator of soil biological activity, was measured using a portable CO2 gas analyzer (EGM-5) (Meza et al., 2018). Three replicate measurements were taken at two locations within each experimental plot, resulting in six measurements per plot and a total of twelve measurements. Soil infiltration was measured using a mini-disc infiltrometer (MDIs; Decagon, 2014). This instrument, suitable for assessing surface soil changes, provides an easy method to estimate soil infiltration rates (Di Prima et al., 2017; Li, et al., 2005). The MDIs features a porous base that allows water to infiltrate the soil. By placing this base in contact with the soil surface and recording water infiltration over a predetermined time interval, soil infiltration capacity was determined.

2.4 Statistical analysis

Statistical analyses were performed to treat, process, and analyse the data obtained, focusing on soil properties associated with infiltration and soil respiration. To determine if there were statistically significant differences between two sets of numerical data, we performed a one-way Analysis of Variance (ANOVA). This method is appropriate for comparing the means of two independent groups. The data was initially stored in an Excel file (sample.xlsx). We utilized the *pandas* library in Python to read and process this data. Specifically, the *read_excel* function was employed with the *openpyxl* engine to ensure compatibility with modern Excel file formats. The script then selected the first two columns (control and treated plots) with all measurements (n = repetitions) from the Excel file, converting them into numerical format using *pd.to_numeric*. Any non-numeric values were coerced to NaN and subsequently removed using *dropna()*. This step ensured that only valid numerical data was used for the ANOVA. The core statistical analysis was performed using the *scipy.stats* library, specifically the *f_oneway* function. This function calculates the F-statistic and the p-value, which are essential for interpreting the results of the ANOVA. To determine statistical significance, we compared the p-value to a predefined alpha level of 0.05. If the p-value was less than 0.05, we concluded that there were statistically significant differences between the two groups. Otherwise, we concluded that there was not enough evidence to support this claim. Then, to examine the relationships between various soil properties, a correlation study was conducted using R Studio software. Pearson's correlation coefficient was used to quantify the strength and direction of linear relationships between variables. This approach facilitated the identification of significant associations among the evaluated properties, contributing to a comprehensive understanding of the interactions influencing soil characteristics.

3 RESULTS

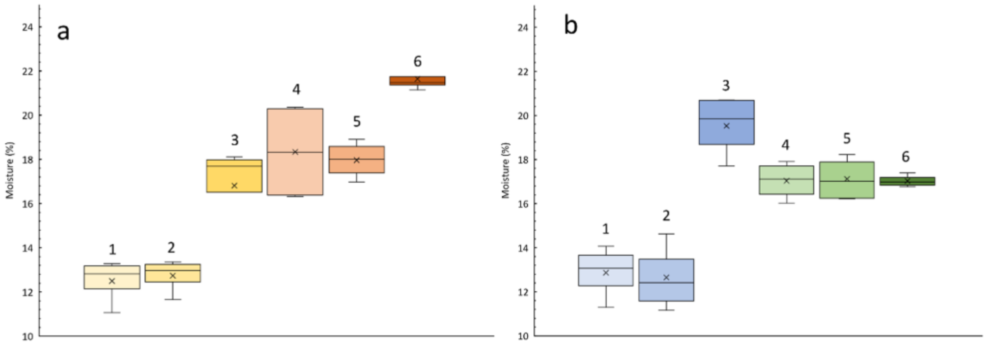
3.1 Soil properties

Figure 3a illustrates the antecedent soil moisture (%) in the earthworm-treated plot, demonstrating a progressive increase over time. Initial average moisture values ranged from 12–14% in the first two samplings, increasing to 18–22% in the final samplings. The standard deviation exhibited moderate variability in the initial samplings, but increased in sampling 4, indicating a wider range of moisture values. Minimum moisture values increased from approximately 12% in the initial samplings to 16–18% in the later samplings, while maximum values increased from 14–15% to over 22%. In the control plot (Figure 3b), initial moisture values were similar (approximately 12–14%), but the increases were less pronounced, stabilizing around 18–20% in the final samplings. The standard deviation was lower compared to the earthworm-treated plot, particularly in the later samplings, as evidenced by the more compact boxplots. Minimum values increased from around 12% to approximately 16%, and maximum values ranged from 14–20%, showing less overall dispersion than the treated plot. The One-way ANOVA did not show significant statistical differences.

Figure 4a illustrates the aggregate stability in the earthworm-treated plot, showing a progressive increase over time. Initial average values were approximately 0.30 in the first two samplings, rising to above 0.40 in the final two samplings. The standard deviation was moderate initially, with increased dispersion in the later samplings, particularly in sampling 6, where minimum values decreased to around 0.20. Maximum values progressed from approximately 0.35 in the initial samplings to about 0.45 in the final samplings. In the control plot (Figure 4b), aggregate stability values were initially higher (sampling 1) than in the earthworm-treated plot, with an average close to 0.40. However, these values showed a slight decrease in subsequent samplings, stabilizing around 0.30–0.35. The standard deviation was generally lower compared to the earthworm-treated plot, except in samplings 3 and 6, where increased dispersion was observed. Minimum values approached 0.20 in the intermediate sampling (3), while maximum values ranged between 0.40 and 0.45 in the first and last samplings. After conducting a One-way ANOVA, F-statistic reached 7.10 with a p value= 0.0091 showing significant statistical differences among the control plot and earthworm-threated one.

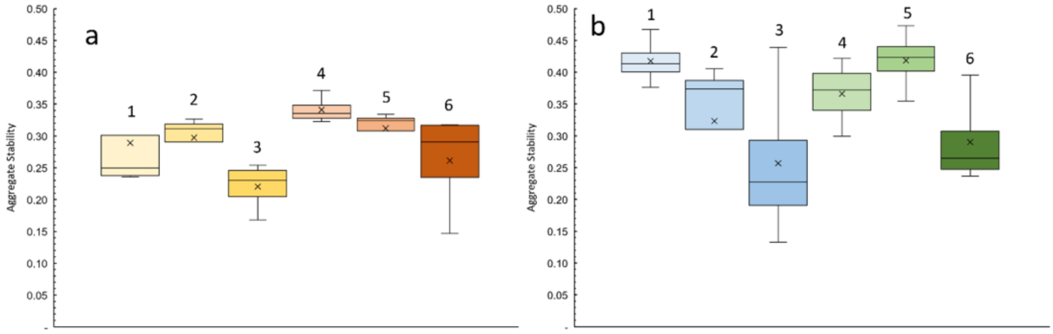
In the earthworm–treated plot (Figure 5a), mean pH values ranged from 8.35 to 8.65. Samples 2 and 4 exhibited higher standard deviations, indicating greater dispersion, whereas samples 1 and 5 showed the most homogeneous values. Maximum pH values reached approximately 8.70 in samples 3 and 5, while the minimum value was approximately 8.10 in sample 4. In the control plot (Figure 5b), mean pH values remained between 8.10 and 8.30, demonstrating less dispersion than in the earthworm-treated plot. The standard deviation was consistently low across all samples, indicating more uniform pH values. Maximum pH values were around 8.30 in samples 5 and 6, while minimum values decreased to 8.00 in samples 2 and 3. After carrying out a One-way ANOVA, F-statistic reached 169.57 with a p value<0.0001 showing significant statistical differences among the control plot and earthworm-threated one.

In the earthworm-treated plot (Figure 6a), mean organic matter content ranged from 5.0% to 6.0%. Samples 2 and 4 exhibited higher standard deviations, indicating greater dispersion, whereas samples 5 and 6 showed less variability. Maximum organic matter content reached approximately 6.5% in samples 1 and 5, while the minimum content decreased to around 4.5% in sample 2. In the control plot (Figure 6b), mean organic matter content ranged from 5.0% to 5.5%. Sample 2 showed the highest standard deviation, reflecting greater dispersion, while samples 4 and 6 exhibited the lowest variability. Maximum organic matter content was close to 6.0% in sample 5, while the minimum content decreased to 4.0% in sample 2. The One-way ANOVA did not show significant statistical differences.



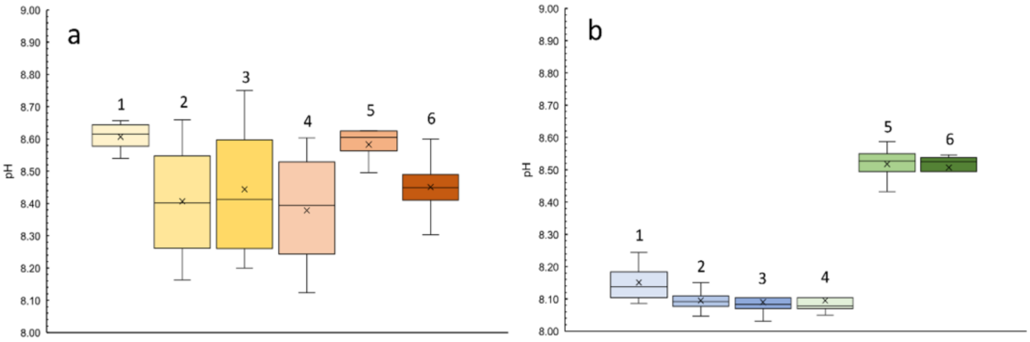
Sampling Number	1	2	3	4	5	6
Date	18/01/2024	30/01/2024	13/02/2024	29/02/2024	13/03/2024	01/04/2024

Fig. 3. Evolution of antecedent soil moisture during the experimental trial. a: box with earthworms´ treatment; b: control plot.



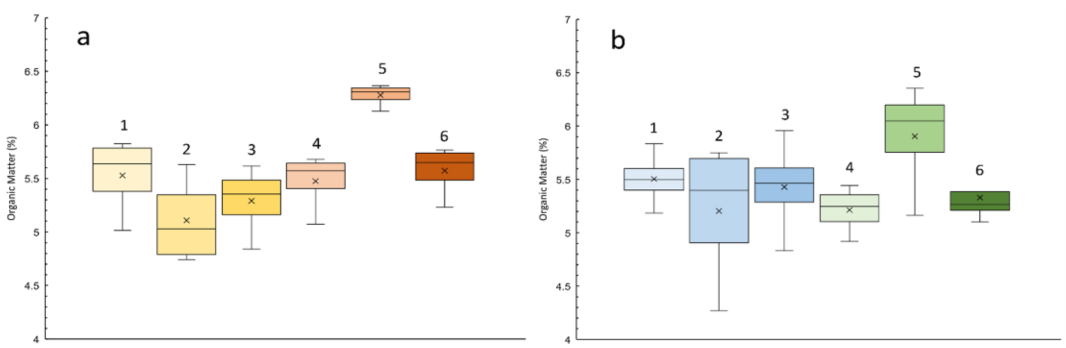
Sampling Number	1	2	3	4	5	6
Date	18/01/2024	30/01/2024	13/02/2024	29/02/2024	13/03/2024	01/04/2024

Fig. 4. Evolution of soil aggregate stability during the experimental trial. a: box with earthworms´ treatment; b: control plot.



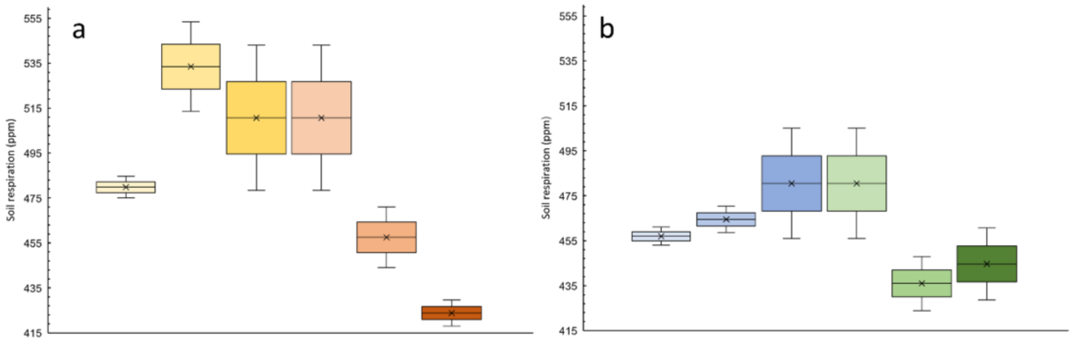
Sampling Number	1	2	3	4	5	6
Date	18/01/2024	30/01/2024	13/02/2024	29/02/2024	13/03/2024	01/04/2024

Fig. 5. Evolution of pH during the experimental trial. a: box with earthworms´ treatment; b: control plot.



Sampling Number	1	2	3	4	5	6
Date	18/01/2024	30/01/2024	13/02/2024	29/02/2024	13/03/2024	01/04/2024

Fig. 6. Evolution of organic matter during the experimental trial. a: box with earthworms´ treatment; b: control plot.



Sampling Number	1	2	3	4	5	6
Date	18/01/2024	30/01/2024	13/02/2024	29/02/2024	13/03/2024	01/04/2024

Fig. 7. Evolution soil respiration during the experimental trial. a: box with earthworms´ treatment; b: control plot.

3.2 Soil respiration

Figure 7a, representing the earthworm-treated plot, shows significant fluctuations in soil respiration. The maximum respiration rate, 555 ppm CO_2 (hereafter referred to as ppm), was observed in measurement 2. The average respiration rate across all measurements was 485 ppm, with a coefficient of variation of 13.1%, which is like the initial measurement before earthworm introduction. The lowest respiration rate, 425 ppm, was recorded in the final measurement on April 1. In the control plot (Figure 7b), soil respiration showed less variability, with a coefficient of variation of 4.77%. The minimum respiration rate, approximately 425 ppm, was observed in measurement 5. The maximum rate, around 505 ppm, was shared between two central measurements. The overall mean respiration rate was 470 ppm, resulting in a difference of 80 ppm between the maximum and minimum values. The One-way ANOVA did not show significant statistical differences.

3.3 Soil infiltration

The introduction of earthworms in vineyard soils within micro-environments significantly enhanced water movement through the soil profile. In Figure 8a (earthworm-treated plot), the infiltration rate started with high values, approximately 7–8 mm h^{-1}, and progressively decreased over time. During the initial time intervals (10 and 100 seconds), data showed greater dispersion, as indicated by the wider whiskers and boxes in the boxplots. However, as time progressed, the infiltration rate stabilized at very low values, close to 0 mm h^{-1}, particularly after 1000 seconds. The initial mean was around 7–8 mm h^{-1}, but decreased significantly over time. The standard deviation also decreased as the infiltration rate stabilized, reflecting lower variability in the final values. During the first 10 seconds, maximum and minimum values ranged widely, between 6- and 8-mm h^{-1}, while in the final intervals (3000 seconds), values were concentrated around 0 mm h^{-1}.

On the other hand, the control plot (Figure 8b) showed a similar initial trend, with a high infiltration rate near 7–8 mm h^{-1}, which also decreased over time. However, the rate reduction appeared more gradual compared to the earthworm-treated plot. In the initial time intervals (10 and 100 seconds), data showed less dispersion than in the earthworm-treated plot, indicating more clustered values. Like the treated plot, the infiltration rate stabilized at low values near 0 mm h^{-1} after 1000 seconds. The initial mean was also around 7–8 mm h^{-1}, with a similar reduction over time. The standard deviation followed a comparable trend to the treated plot, being higher in the initial times and decreasing as values stabilized. Maximum and minimum values in the early intervals also ranged between 7 and 8 mm h^{-1}, while in the later intervals, as in the treated plot, they were concentrated around 0 mm h^{-1}. The One-way ANOVA did not show significant statistical differences.

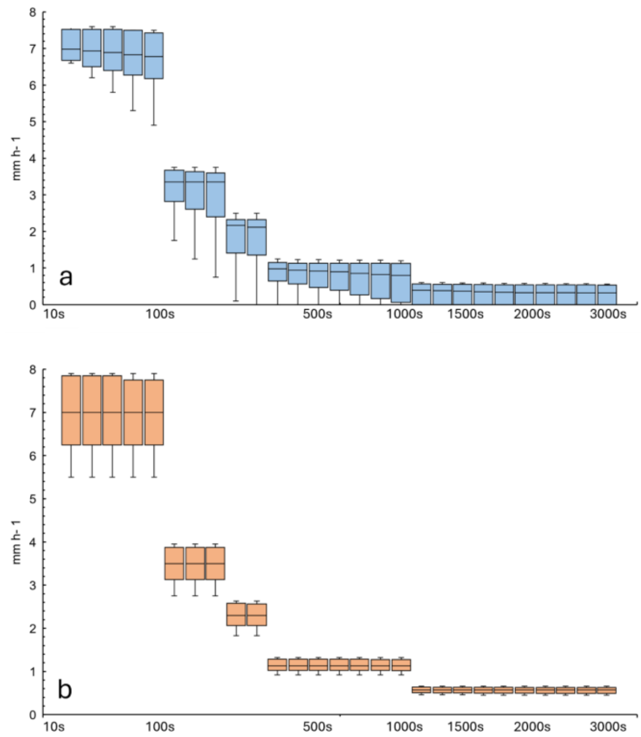


Fig. 8. Average soil infiltration rates per interval considering control (upper) and treated (below) plots.

3.4 Correlation matrix analysis

Figure 9 presents correlation matrices examining the relationships among soil properties before and after earthworm introduction. The matrix for the initial sample reveals baseline correlations, while matrix 6 illustrates how these relationships evolved during the study period. Significant differences were observed in the correlations among moisture, pH, organic matter, and soil stability, reflecting potential changes in soil dynamics due to environmental conditions, soil management, natural processes, or earthworm activity.

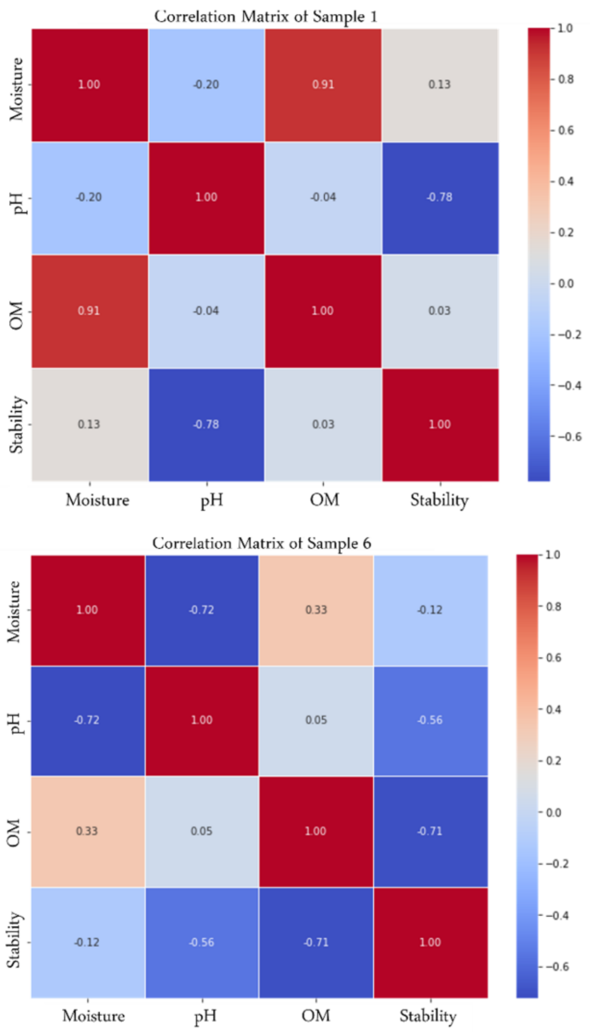


Fig. 9. Analysis of correlation among soil properties.

In the initial sample matrix, a strong positive correlation (0.91) was evident between moisture and organic matter, indicating a close association between these properties. The correlation between moisture and soil stability was weak (0.13), suggesting a less significant relationship. pH showed a moderate negative correlation with moisture (–0.20) and a strong negative correlation with soil stability (–0.78), indicating that higher pH may be associated with lower soil stability. Organic matter showed weak correlations with pH (–0.04) and stability (0.03), suggesting minimal interaction between these properties in the initial sample. In summary, the correlation matrices reveal a noticeable shift in the interactions among the analysed variables between the initial and final sampling dates. The observed decrease in the correlation between moisture and organic matter, coupled with variations in the correlations among pH, stability, and other soil properties, suggests that external factors or altered soil dynamics have influenced these relationships.

4 DISCUSSION

This study has demonstrated that the introduction of earthworms into micro-ecosystems (~1 m²) of soils originating from vineyards can significantly alter some key soil properties, particularly those related to respiration and infiltration like as pH and aggregate stability. These findings align with previous studies conducted under similar conditions in other European vineyards (Vršic et al., 2021). The introduction of earthworms into Mediterranean vineyard soils, which often face challenges such as low organic matter, high compaction, and runoff, represents a crucial strategy for soil regeneration and environmental impact mitigation (Bogunovic, et al., 2020). Earthworms function as biological agents that enhance organic matter through long-term humus production while simultaneously promoting the redistribution of soil particles, leading to the formation of stable aggregates (Józefowska et al., 2021; Ketterings, et al., 1997). This process, advocated decades ago (Dutt, 1948), is essential for improving aggregate stability, which in turn enhances soil resistance to erosion and optimizes water and nutrient storage (Guo et al., 2024). Moreover, the burrows created by earthworms serve a dual function (Villarreal et al., 2024): not only do they increase infiltration capacity, but they also influence soil gas dynamics by creating microenvironments that facilitate CO_2 fixation, thereby reducing net emissions to the atmosphere.

The biological activity of earthworms also has complex implications for parameters such as soil pH and respiration (Thomas et al., 2020). While immediate changes in pH appear (as our statistical analysis demonstrated), research suggests that more pronounced effects emerge over time, likely due to the accumulation of organic compounds and interactions between earthworm-associated microorganisms (Fisk, et al., 2004). In terms of soil respiration, the initial increase observed in this study indicates heightened microbial activity driven by the decomposition of fresh organic matter (Capowiez et al., 2021). However, the subsequent decline in respiration rates suggests a shift toward a more stabilized system, where organic matter undergoes gradual stabilization (Barthod et al., 2020).

Regarding infiltration, this study found significant differences in the average, maximum and minimum values between treated and control plots, although it was not reflected by the ANOVA one-way (like the soil respiration). The initial hypothesis that earthworm burrows would enhance infiltration was confirmed (Capowiez et al., 2021). The infiltration rates in the biologically treated plot were consistently higher than in the control plot. Moreover, as earthworms remained active in the area over time, infiltration capacity progressively improved. Notably, from the fourth measurement onward, after the 9–10-minute interval, all water in the infiltrometer was consistently absorbed in the treated plot–a pattern not observed in earlier measurements or in the control plot. This result underscores the capacity of earthworms to improve soil infiltration rates and water retention. To visually illustrate these changes, a video was created comparing infiltration rates on two different dates in the treated plot (https://youtu.be/nXmMSS1x__w?si=f_hx2wkecHBcKiYL).

These findings highlight the potential of earthworms not only to rehabilitate degraded soils but also to contribute to sustainable agricultural practices that balance productivity with carbon emissions mitigation. However, despite these promising results, several challenges remain in maximizing the positive impact of earthworms on degraded Mediterranean vineyard soils. One key issue is the scalability and applicability of these findings across diverse vineyard conditions. While micro-ecosystem trials provide valuable insights, replicating these results in real-field conditions–where soil dynamics, climate variability, and agricultural management practices are more complex–remains a challenge (Kinnell, 2022, Rodrigo-Comino et al., 2017). Future research should investigate how factors such as tillage practices, pesticide use, and vineyard-specific soil characteristics influence the effectiveness of earthworm-driven soil improvements.

Another critical consideration is the long-term sustainability of using earthworms from both an ecological and economic perspective. Earthworms are cost-effective compared to chemical soil amendments (a group of 100 Lumbricus terrestris costs approximately €50) and have the ability to reproduce, meaning that only an initial introduction is needed to establish a self-sustaining population. However, while improvements in aggregate cohesion or pH, or slowly in infiltration, soil respiration and organic matter accumulation are encouraging, further research is needed to determine whether these benefits persist without frequent reintroductions at long-term periods. It is necessary to check if the ANOVA one-way increasing the time and number of measurements would show significant changes. This would demonstrate how well earthworms integrate into vineyard soil ecosystems and whether factors such as intensive agricultural activity, predation by birds and mammals, or environmental fluctuations might counteract their beneficial effects. The latter consideration is particularly relevant in the context of EU policies promoting biodiversity in agricultural landscapes, especially given that the vineyard in this study is located just 3 km from a designated wetland (Arias-García, 2019).

It also remains to be determined whether the introduction of earthworms is compatible with other regenerative approaches and nature-based solutions, such as the use of pruning residues. Earthworms should be considered as part of an integrated soil management strategy that incorporates complementary practices such as cover cropping, crop rotation, and biofertilizer application. Evaluating potential synergies or conflicts between these methods and earthworm activity is crucial for designing effective, sustainable strategies. Moreover, while evidence suggests that earthworms can enhance CO_2 sequestration, as observed in this study, it is essential to establish standardized protocols to measure these long-term effects accurately. This will allow for a more comprehensive assessment of their role in climate change mitigation across different environmental contexts.

Additionally, the genetic and functional diversity of earthworm species must be taken into account. Not all earthworm species exert the same influence on soil properties. Future studies should investigate which species are most effective for addressing specific soil issues, such as compaction, low organic matter, or unbalanced pH, and whether their introduction is viable across different soil types. Addressing these challenges will require an interdisciplinary approach that integrates soil biology, ecology, agricultural engineering, and climate science to develop scalable, long-term solutions for improving vineyard soil health.

Fig. 10. Examples of earthworms in the treated plots and reproduction during the trial experimental period.

5 CONCLUSIONS

The analysis and discussion of the results confirm the initial hypotheses of this research. Despite the study's short duration, it was sufficient to observe initial changes in key soil properties resulting from earthworm activity. Earthworms demonstrably improved soil moisture levels and organic matter content. While the impact on aggregate stability was less pronounced, earthworms effectively enhanced soil respiration rates and contributed to CO_2 sequestration. Notably, they improved both the capacity and rate of water infiltration. Ultimately, this research highlights the significant potential of earthworms to enhance soil health and key soil properties. This study paves the way for future research, which should prioritize long-term studies in real-world settings to assess the scalability of these small-scale findings to large-scale applications, enabling earthworms to influence extensive land areas.

Aknowledgements. We would like to express our gratitude to the owners of Bodegas Calvente, located in the municipality of Villamena (Granada, Spain), and to our colleagues who assisted with the field research. This work was supported by a 2021 Leonardo Grant for Researchers and Cultural Creators from the BBVA Foundation, for which we are deeply grateful. We also acknowledge the support from projects granted by the University of Granada within the Plan Propio: (i) PP2022.EI-01, "Caracterizando la degradación ambiental en el viñedo granadino. Un enfoque multidisciplinar a largo plazo utilizando parcelas experimentales y muestreos poblacionales"; 1 (ii) PPJIA2022-58, "Caracterización hídrica del suelo en viñedos para la optimización de recursos agrícolas y ambientales"; and (iii) Proyectos Innovadores 2022–2025 de Grupos Operativos AEI-agri (Andalucía): "Creación de una herramienta DSS (Decision Support System) para el manejo sostenible del suelo en viticultura.

RERENCES

Adhikari, K., Hartemink, A. E., 2016. Linking soils to ecosystem services — A global review. Geoderma, 262, 101–111. https://doi.org/10.1016/j.geoderma.2015.08.009

Amézketa, E., 1999. Soil aggregate stability: a review. Journal of sustainable agriculture, 14(2-3), 83-151.

Arias-García, J., 2019. Propuesta metodológica para la identificación, caracterización y cualificación de los paisajes: la cuenca endorreica de Padul (Andalucía) como caso de estudio. BAGE. Boletín de la Asociación Española de Geografía, (80). https://dialnet.unirioja.es/servlet/artic

Barley, K.P., 1959. The influence of earthworms on soil fertility. I. Earthworm populations found in agricultural land near Adelaide. Australian Journal of Agricultural Research, 10(2), 171-178. https://doi.org/10.1071/AR9590171

Barthod, J., Dignac, M.-F., Le Mer, G., Bottinelli, N., Watteau, F., Kögel-Knabner, I., Rumpel, C., 2020. How do earthworms affect organic matter decomposition in the presence of clay-sized minerals? Soil Biology and Biochemistry, 143, 107730. https://doi.org/10.1016/j.soilbio.2020.107730

Bendezú Felices, F. J., 2022. Study of nutrient concentration and absorption in the cultivation of the Crimsson Seedless grape variety in organic drip irrigation production in Villacurí. https://acortar.link/Y6YLv5

Bhadauria, T., Saxena, K. G., 2010. Role of earthworms in soil fertility maintenance through the production of biogenic structures. Applied and Environmental Soil Science, 816073. https://doi.org/10.1155/2010/816073

Bogunovic, I., Telak, L. J., Pereira, P., 2020. Experimental comparison of runoff generation and initial soil erosion between vineyards and croplands of Eastern Croatia: A case study. Air, Soil and Water Research, 13, 1–10. https://doi.org/10.1177/1178622120928323

Bünemann, E. K., Bongiorno, G., Bai, Z., Creamer, R. E., De Deyn, G., de Goede, R., Fleskens, L., Geissen, V., Kuyper, T. W., Mäder, P., Pulleman, M., Sukkel, W., van Groenigen, J. W., Brussaard, L., 2018. Soil quality – A critical review. Soil Biology and Biochemistry, 120, 105–125. https://doi.org/10.1016/j.soilbio.2018.01.030

Capowiez, Y., Sammartino, S., Keller, T., Bottinelli, N., 2021. Decreased burrowing activity of endogeic earthworms and effects on water infiltration in response to an increase in soil bulk density. Pedobiologia, 85–86, 150728. https://doi.org/10.1016/j.pedobi.2021.150728

Carvajal, J., 2024. Cambios en la infiltración. Youtube. https://youtu.be/nXmMSS1x__w?si=W-Eet0l7-dmQQ1gc

Conrad, L., Hörl, J., Henke, M., Luick, R., Schoof, N., 2022. Sheep in the vineyard: Suitability of different breeds and potential breeding objectives. Animals, 12(19), 2575. https://doi.org/10.3390/ani12192575

Di Prima S, Bagarello V, Lassabatere L, Angulo-Jaramillo, R., Bautista, I., Burguet, M., Cerdà, A., Iovino, M., Prosdocimi, M., 2017. Comparing Beerkan infiltration tests with rainfall simulation experiments for hydraulic characterization of a sandy-loam soil. Hydrological Processes. 31: 3520–3532. https://doi.org/10.1002/hyp.11273

Domínguez, J., Aira, M., Gómez-Brandón, M., 2009. The role of earthworms in the decomposition of organic matter and the nutrient cycle. AEET. https://www.revistaecosistemas.net/index.php/ecosistemas/article/view/61/58

Dutt, A. K., 1948. Earthworms and soil aggregation. Agronomy Journal, 40(5), 241–249. https://doi.org/10.2134/agronj1948.00021962004000050004x

Edwards, C. A., Arancon, N. Q., 2022. The role of earthworms in organic matter and nutrient cycles. In Biology and Ecology of Earthworms (pp. 233-274). Springer. https://doi.org/10.1007/978-0-387-74943-3_8

Eisenhauer, N., Scheu, S., 2008. Earthworms as drivers of the competition between grasses and legumes. Soil Biology and Biochemistry, 40(10), 2650-2657. https://doi.org/10.1016/j.soilbio.2008.07.010

Eldridge, D. J., Ding, J., Travers, S. K., 2022. A global synthesis of the effects of livestock activity on hydrological processes. Ecosystems, 25, 1780–1791. https://doi.org/10.1007/s10021-022-00746-9

Fernández-Gálvez, J., Gálvez, A., Peña, A., Mingorance, M. D., 2012. Soil hydrophysical properties resulting from the interaction between organic amendments and water quality in soils from Southeastern Spain—a laboratory experiment. Agricultural Water Management, 104, 104-112.

Fisk, M., Fahey, T., Groffman, P., Bohlen, J.P., 2004. Earthworm invasion, fine-root distributions, and soil respiration in North Temperate Forests. Ecosystems, 7, 55–62. https://doi.org/10.1007/s10021-003-0130-3

Fitzpatrick, R. W., 2009. Soil: forensic analysis. Wiley encyclopedia of forensic science. Chichester: John Wiley & Sons, 2377-88.

Grinhut, T., Hadar, Y., Chen, Y., 2007. Degradation and transformation of humic substances by saprotrophic fungi: Processes and mechanisms. Fungal Biology Reviews, 21(4), 179–189. https://doi.org/10.1016/j.fbr.2007.09.003

Guanche, A., 2015. Las Lombrices y La Agricultura. Cabildo Insular de Tenerife. Oficina Extensión Agraria y Desarrollo Rural – La Orotava

Hole, F. D., 1981. Effects of animals on soil. Geoderma, 25(1-2), 75–112. https://doi.org/10.1016/0016-7061(81)90008-2

Instituto Geográfico Nacional. 2022. Plan Nacional de Ortofotografía Aérea (PNOA). Centro de Descargas del CNIG. https://centrodedescargas.cnig.es

IUSS Working Group WRB, 2022. World Reference Base for Soil Resources: International soil classification system for naming soils and creating legends for soil maps (4ª ed.). International Union of Soil Sciences (IUSS).

Jozefowska, A., Woźnica, K., Sokołowska, J., Sochan, A., Zaleski, T., Ryżak, M., Bieganowski, A., 2021. Stability of aggregates made by earthworms in soils with organic additives. Agronomy, 11(3), 421.

Kassam, A., Friedrich, T., Derpsch, R., Pretty, J., 2016. Conservation agriculture: Sustainable farming under changing climate. *Soil and Tillage Research, 155*, 1-6. https://doi.org/10.1016/j.still.2015.10.002

Ketterings, Q. M., Blair, J. M., Marinissen, J. C. Y., 1997. Effects of earthworms on soil aggregate stability and carbon and nitrogen storage in a legume cover crop agroecosystem. Soil Biology and Biochemistry, 29(3–4), 401–408. https://doi.org/10.1016/S0038-0717(96)00102-2

Kibblewhite, M. G., Ritz, K., Swift, M. J., 2007. Soil health in agricultural systems. Philosophical Transactions of the Royal Society B: Biological Sciences, 363(1492), 685–701. https://doi.org/10.1098/rstb.2007.2178

Kinnell, P. I. A., 2022. Insights into the sediment transport processes operating in rain-impacted flows. CATENA, 217, 106452. https://doi.org/10.1016/j.catena.2022.106452

Lal, R., Kimble, J. M., Follett, R. F., Cole, C. V., 2004. Soil carbon sequestration impacts on global climate change and food security. Science, 304(5677), 1623-1627. https://doi.org/10.1126/science.1097396

Li, X. Y., González, A., Solé-Benet, A., 2005. Laboratory methods for the estimation of infiltration rate of soil crusts in the Tabernas Desert badlands. Catena, 60(3), 255-266

Liao, J., Ni, J., Zou, X., Chen, H. Y. H., Delgado-Baquerizo, M., Li, Y., Ren, T., Shi, K., Ruan, H., 2024. Earthworms regulate soil microbial and plant residues through decomposition. Geoderma, 450. https://doi.org/10.1016/j.geoderma.2024.117040

Meza, F. J., Montes, C., Bravo-Martínez, F., Serrano-Ortiz, P., Kowalski, A. S., 2018. Soil water content effects on net ecosystem CO2 exchange and actual evapotranspiration in a Mediterranean semiarid savanna of Central Chile. Scientific reports, 8(1), 8570

Minea, G., Mititelu-Ionuș, O., Gyasi-Agyei, Y., Ciobotaru, N., Rodrigo-Comino, J., 2022. Impacts of grazing by small ruminants on hillslope hydrological processes: A review of European current understanding. Water Resources Research, 58(2), e2021WR030716. https://doi.org/10.1029/2021WR030716

Moraine, M., Boiron, G., de Sartre, X. A., Gasselin, P., 2024. June). Optimizing territorial resources to support agroecological transitions: which levers to adapt to climate change in livestock systems in South of France. In 15th IFSA Conference, Systemic Change For Sustainable Future, Trapani, Italy.

Panagos, P., Broothaerts, N., Ballabio, C., Orgiazzi, A., De Rosa, D., Borrelli, P., Liakos, L., Vieira, D., Van Eynde, E., Arias Navarro, C., Breure, T., Fendrich, A., Köninger, J., Labouyrie, M., Matthews, F., Muntwyler, A., Martin

Jimenez, J., Wojda, P., Yunta, F., Marechal, A., Sala, S., Jones, A., 2024. How the EU Soil Observatory is providing solid science for healthy soils. European Journal of Soil Science. https://doi.org/10.1111/ejss.13507

Porta Casanellas, J., López-Acevedo Reguerín, M., Roquero de Laburu, C., 2003. Edafología: para la agricultura y el medio ambiente (3ª ed.). Ediciones Mundi-Prensa

Publicaciones de la FAO, 2013. Rainwater harvesting and storage. https://www.fao.org/4/i3247s/i3247s.pdf

Publicaciones de la FAO. (n.d.). Soil structure. https://www.fao.org/fishery/static/FAO_Training/FAO_Training/General/x6706s/x6706s07.htm

Rodrigo-Comino, J., Brings, C., Iserloh, T., Casper, M. C., Seeger, M., Senciales, J. M., Brevik, E. C., Ruiz-Sinoga, J. D., Ries, J. B., 2017. Temporal changes in soil water erosion on sloping vineyards in the Ruwer-Mosel Valley: The impact of age and plantation works in young and old vines. Journal of Hydrology and Hydromechanics, 65(4), 402-409. https://doi.org/10.1515/johh-2017-0022

Schulte, E. E., Hopkins, B. G., 1996. Estimation of soil organic matter by weight loss-on-ignition. En F. R. Magdoff, M. A. Tabatabai, & E. A. Hanlon Jr. (Eds.), SSSA Special Publications (Cap. 3). Soil Science Society of America. https://doi.org/10.2136/sssaspecpub46.c3

Siddiqui, S. A., Harahap, I. A., Osei-Owusu, J., Saikia, T., Wu, Y. S., Fernando, I., Perestrelo, R., Câmara, J. S., 2024. Bioconversion of organic waste by insects – A comprehensive review. Process Safety and Environmental Protection. https://doi.org/10.1016/j.psep.2024.04.122

Smith, E. J., Ryan, J. L., Lopresti, S. A., Haghnazari, D. B., Anderson, K. A., Lipson, S. J., Johnson, C.E., Silver, L.W., Saunders, C.J., 2023. Mechanisms of carbon dioxide detection in the earthworm Dendrobaena veneta. Frontiers in Ecology and Evolution, 11, 1202410.

Swift, M. J., et al. (1979). Decomposition in terrestrial ecosystems. Studies in Ecology, 5. https://acortar.link/uDZXKQ

Thomas, E., Viswanathan, S. P., Kurien, V. T., Thomas, A. P., 2020. The potential of earthworms in soil carbon storage: A review. Environmental and Experimental Biology, 18, 61–75.

Villarreal, R., Lozano, L. A., Salazar, M. P., Polich, N. G., Bellora, G. L., Ellerbrock, R. H., Barbosa, L. A. P., Gerke, H. H., Soracco, C. G., 2024. Hydraulic characterization of earthworm macropore surfaces using miniaturized infiltration data. Soil and Tillage Research, 236, 105954. https://doi.org/10.1016/j.still.2023.105954

Vršič, S., Breznik, M., Pulko, B., Rodrigo-Comino, J., 2021. Earthworm abundance changes depending on soil management practices in Slovenian vineyards. Agronomy, 11(6), 1241.

Received 21 December 2024
Accepted 21 March 2025