

## RESEARCH ARTICLE OPEN ACCESS

# Does an Increase in Number of Deep Burrowing Earthworms Enhance Subsurface Nutrient Losses?

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**Received:** 29 March 2025 | **Revised:** 25 January 2026 | **Accepted:** 2 February 2026

**Keywords:** bioturbation | boreal climate | earthworm inoculation | *Lumbricus terrestris* | macropores | nitrogen | no-till | phosphorus | preferential flow | subdrainage

## ABSTRACT

Deep burrowing earthworms *Lumbricus terrestris* can be abundant along subdrain trenches, creating macropores that may serve as preferential flow paths for particles and agrochemicals to subdrains. We examined *L. terrestris* abundance, subdrain water discharge, and nutrient loss trends during 2002–2022 in an arable no-till clay soil with an emerging *L. terrestris* density gradient. Additionally, we analyzed subdrain water discharge during heavy rainfall events and traced material movement from topsoil to subsurface drains utilizing a <sup>137</sup>Cs marker. Between 2003 and 2009, inoculated *L. terrestris* started to establish at the upper edge of the field site and successive samplings revealed gradual population growth and colonization downslope. Along the 134 m field strips ( $N=2$ ), a *L. terrestris* mean burrow count in 2022 reached  $37\text{ m}^{-2}$  (range 29–54) with a mean individual count of  $27\text{ m}^{-2}$  (13–54) at the upper edge close to the inoculation area, but on average only 5 burrows (0–8) and 2 individuals  $\text{m}^{-2}$  (0–8) were recorded at the lower edge. Simultaneously with *L. terrestris* spreading, water, sediment and nutrient discharges via subsurface drains nearly doubled. Despite these concomitant trends, statistical analyses did not support the hypothesis that long-term drainage discharges were coupled with the *L. terrestris* gradient. Neither were storm water discharges, nor topsoil (specifically <sup>137</sup>Cs) migration to drains in agreement with the *L. terrestris* gradient. Long-term increase in discharges were likely due to increased precipitation outside the growing season (2002–2022 trend for Oct–Apr precip.  $+2.4\text{ mm year}^{-1}$ ) and increasing winter/spring temperatures (2002–2022 trend for Jan–Mar temp.  $+0.12\text{ deg. year}^{-1}$ ). Additionally, different backfill materials of the upper (topsoil) and lower (wood chips) parts of the field may have affected the results. Under the given environmental conditions and cultivation, the agronomic benefits of *L. terrestris* activity do not appear to come with a cost of increased subdrain leaching.

## 1 | Introduction

In a Boreal climate, drainage of arable land is needed for conveying excess water from the fields to ensure adequate crop growth conditions and trafficability. Hence, a vast majority of the agricultural fields on fine-textured mineral soils in Finland are equipped with

a subsurface drainage system. Particularly in clayey subdrained fields, the drainage discharge forms a major water balance component (Jin and Sands 2003; Koivusalo et al. 2017). Preferential flow phenomenon is important in the hydrology of these soils, and a major share of water flow to the subdrains can occur via preferential flow paths (e.g., Warsta et al. 2013).

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## Highlights

- Impacts of deep burrowing earthworms on subdrain discharge and leaching studied in field conditions.
- In long-term data, the increase of earthworm density had no discernible effect on discharge or leaching.
- Analyses of storm-event data revealed no earthworm impact on subdrain discharge.
- In Boreal heavy clay under no till, deep burrowing earthworms did not increase the risk of leaching.

Preferential flow is largely controlled by the soil pore structure, that is the arrangement of soil pore space. In clayey soils, macropores with a diameter  $\geq 300 \mu\text{m}$ , consisting mainly of biopores and desiccation cracks, allow rapid flow through the soil profile, largely allowing the percolating water to bypass the soil matrix comprising smaller pores (e.g., Jarvis 2007). Layers of coarser material within the soil matrix can also induce preferential flow (e.g., Salo et al. 2017). In addition to soil characteristics such as texture and carbon content (Klöffel et al. 2024), the formation of the soil structure relates importantly to biopores resulting from root growth and action of soil macrofauna, particularly deep burrowing earthworms (e.g., Meurer et al. 2020).

Under ponded conditions in particular, a minuscule soil volume consisting of larger voids and pores may deliver almost all the percolation water (Watson and Luxmoore 1986). Macropore flow has been identified as responsible for rapid translocation of solutes, soil colloids, and particles from the soil surface to subsurface drains or groundwater (Pilgrim et al. 1978; Jacobsen et al. 1997; Williams et al. 2016). Colloid- and particle-facilitated transport means that strongly soil-sorbing chemicals and elements may also migrate to drainage systems (Uusitalo et al. 2001). Macropore-mediated solute and particle transport are affected by given environmental factors, including soil texture and weather, and by factors that can be managed. Based on stable oxygen isotopes, Williams et al. (2016) detected a higher proportion of newly precipitated water in tile drainage discharges after rainstorms when tillage had been omitted, as compared to disk-tilled soil where continuity of macropores was disturbed by tillage. Surface-applied P fertilizer also migrated in no-till soil to a higher degree as dissolved P after subsequent storm events, which was interpreted to result from direct leaching from the soil surface to drainage pipes.

While subdrainage design and function have been widely studied, there is limited knowledge on the implications of bioturbation by macrofauna for soil structure formation and water and solute movement in subdrained soils. This is of practical importance as soil structure is a dynamic property. Over time, soil structure, drainage efficiency and consequently water balance can be adversely affected by farming operations (e.g., Keller et al. 2019) and drain clogging (e.g., Nousiainen et al. 2015; Turtola and Paajanen 1995). It has been shown that structural feedback of macrofauna and other factors can impact long-term field water balance (Jarvis et al. 2024) and small changes in the pore structure can have a large impact on the hydraulic conductivity of clay soils (e.g., Soenne et al. 2023).

In agricultural soils a transition from moldboard plowing to non-inversion tillage or no-till—or when rotation with lowered tillage frequency is adopted—typically leads to a marked increase of deep burrowing earthworms such as the epi-aneic species *Lumbricus terrestris* L. (Lindahl et al. 2009; Briones and Schmidt 2017). These species benefit when their burrows are not disturbed by inversion tillage and when their important food source, plant residues on the soil surface, remain available. The vertical burrows of *L. terrestris* open at the soil surface, are wide ( $\varnothing$  5–10 mm) and continuous, and may reach to a depth greater than 1 m. When a clay soil is moist and swollen, the burrows remain open unlike physically created macropores such as desiccation cracks. When the soil is not disturbed, a particular burrow may remain occupied for many years (Potvin and Lilleskov Potvin and Lilleskov 2016) and an abandoned burrow can retain its structure for tens of years in the subsoil (VandenBygaert et al. 1998). Together these characteristics render *L. terrestris* burrows important conduits of rain and melt water, and dissolved substances into subsoil (Shipitalo and Le Bayon 2004; Jarvis 2007; Hallam and Hodson 2021).

In subdrained field soils deep earthworm burrows are of special hydrological interest. Urbánek and Doležal (1992) noticed that earthworm burrows can be in direct contact with subdrain tiles and increase the permeability of trench backfills. Using a different approach, Shipitalo and Gibbs (2000) came to the same conclusion. Their study also indicated that the flow of water into subdrains along *L. terrestris* burrows contributed to observed rapid leaching of slurry into waterways through the subdrain system. Some of these findings were corroborated in clayey fields of S-W Finland, where it was found that *L. terrestris* burrows can have direct contacts with subdrains (Nuutinen and Butt 2003) and contribute to trench backfill permeability and elevated infiltration rates above the subdrain lines (Shipitalo et al. 2004). In the same region, the densities of *L. terrestris* can be especially high at the subdrain lines (Nuutinen et al. 2001, 2011), a pattern which was not seen in field soils studied in Ohio by Shipitalo and Gibbs (2000) and in Quebec by Diop et al. (2023).

In S-W Finland, the dominantly clayey arable soils are mainly used for the production of cereals and reduced tillage and no-till have been widely adopted. Growing interest in regenerative agriculture is likely to further reduce mechanical soil disturbance (Storkey et al. 2024). This points to a growing importance of *L. terrestris* for the structure and hydrology of the region's field soils, as the species is also the second most common earthworm in agricultural soils of Finland (Nieminen et al. 2011). The aim of this study was to gain understanding on the impacts which an increasing *L. terrestris* abundance may have on subdrain discharge and leaching of nutrients in heavy clay under no-till management. The study utilized a unique long-term field experiment where data on settlement and spread of an introduced *L. terrestris* population could be coupled with follow-up data on subdrain discharge and leaching. The specific research questions addressed were: (1) Does the gradual increase in *L. terrestris* density affect subdrain discharge and leaching in the long run? and (2) Is the abundance of *L. terrestris* and their burrows related to the subdrain discharge at exceptionally heavy rainfall events? Our study hypothesis was that the increase of *L. terrestris* abundance would increase subdrain discharge and off-site nutrient losses.

## 2 | Materials and Methods

### 2.1 | Field Site

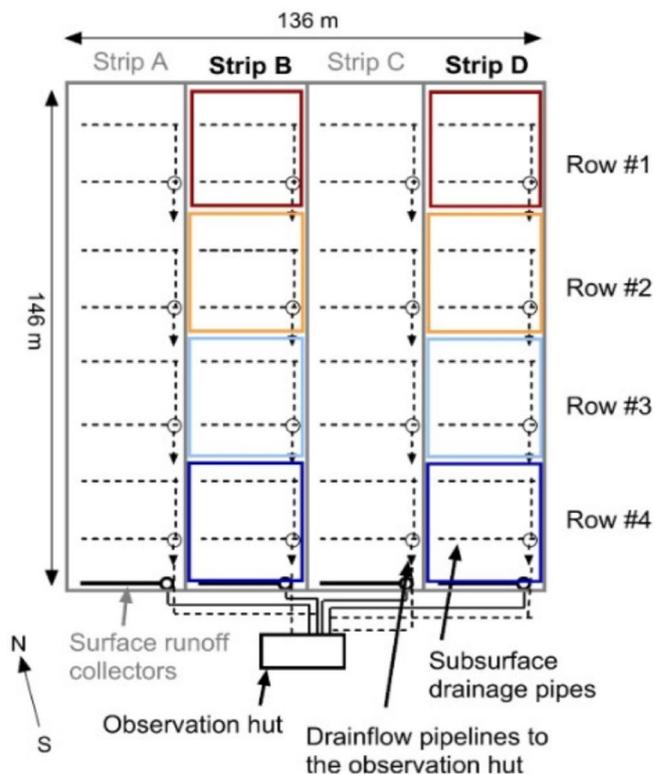
#### 2.1.1 | Field and Drainage Design

The study was conducted on an experimental arable field established in 1975 to study erosion and nutrient leaching. The field (Kotkanoja) is in Jokioinen, in southwestern Finland (60° 49' N, 23° 30' E, about 100 m a.s.l.) and has a mean slope of 2% (range 1%–4%). The average contents of clay, silt, and sand in the topsoil (0–30 cm) are 60%, 16%, and 24%, respectively, and the soil is classified as Protovertic Luvisol (Honkanen et al. 2021). The long-term (1991–2021) annual mean temperature at the site is +5.2°C, and the mean annual precipitation 621 mm. The soil is typically frozen and under snow cover from December to March/April. The annual precipitation during 2002–2022 (the period of this study) is shown in Figure S1, and more specific patterns of precipitation and temperature over the study period in Figure S2.

The field consists of four 0.5-ha field strips (labeled A, B, C and D) that are isolated from each other and the surrounding environment by a plastic curtain extending 1 m below the ground surface (the depth of the drainage pipes) and a 0.3 m high ley-growing ground ridge (Figure 1). All four field strips are divided lengthwise into four equal parts, forming a total of 16 plots (Figure 1). Each plot is separately subdrained by two parallel suction pipes, 16.5 m apart. In this study, we used data of strips B and D, where no inversion tillage has been applied since 1991 (with one exception in plot D, described below) and which have distinct patterns of *Lumbricus terrestris* spread and distribution following their introduction (Section 2.1.2). The excluded strips (A and C) were annually ploughed before the period of this study and again from 2008 onwards, which kept *L. terrestris* practically absent from them.

In 1991, the drainage system of the field was renovated by installing plastic drainage pipes 0.3 m apart from the old tile drainpipes. New drainage pipes were installed at the bottom of 1-m-deep trenches and covered with a 15 cm layer of gravel. At five points of each suction pipe, a thicker layer of gravel (40–50 cm) was placed on top to improve the access of water to the drainage pipe. At the upslope half of the field (rows #1 and #2; Figure 1) trenches were backfilled with topsoil, while at the downslope half (rows #3 and #4; Figure 1) wood chips were used. Wood chips were used as backfill at the lower end because heavy rains interfered with the drain installation and soil left drying on surface was no longer suitable as backfill material (Turtola and Paajanen 1995). The drainage water from each plot is conducted separately to an observation hut (Figure 1).

After the drainage renovation, two of the field strips (B and D) were not ploughed. At the start of the period covered by this study, the field was maintained in green fallow (2002–2007). Thereafter, strips B and D were cultivated with cereals using direct drilling, except that in summer 2018, strip D was ploughed once and sown with rapeseed. After the rapeseed harvest in 2019, this strip returned to no-till management with cereals. Details of crop rotations and farming measures since 2002 are presented in Table S1.



**FIGURE 1** | The experimental set-up at Kotkanoja site. The study involved the no-till plots of the field, named here strip B and D. In the analyses of the data, the strips were divided into four rows corresponding with their division in subdrainage plots (rectangles), and with the temporal and spatial gradient of *L. terrestris* abundance. Inoculation of *L. terrestris* took place in 1996 at the upper end of row #1. The marking of the subdrainage plots with rectangles is illustrative and does not correspond exactly with their actual size and position. The colour code of rows is used in all illustrations. For detailed description of the experiment, refer to the text.

#### 2.1.2 | Earthworm Surveys in Kotkanoja and the Inoculation of *L. terrestris*

In autumn 1988, earthworm extraction with formalin was undertaken within four 0.5 m<sup>2</sup> quadrats in all 16 plots of the field (Luke, unpublished). The earthworms obtained were shallow-burrowing (endogeic) *Aporrectodea caliginosa* (Sav.) and the residue layer dwelling (epigeic) *Lumbricus rubellus* (Hoffm.), both present at low densities, but no *L. terrestris* were found. At the same time, presence of *L. terrestris* was investigated in the permanent grass zones surrounding the field by searching for middens and by shallow excavations to seek burrows. Neither of these surveys revealed any signs of *L. terrestris*. In autumn 1996, two weeks before *L. terrestris* inoculation in the field, the absence of the species in the field was confirmed by negative results from chemical extraction at the up-slope part of the field, inside the field and at the boundary grass belt (Nuutinen et al. 2006).

Inoculation of *L. terrestris* was done in October 1996 in the up-slope end of the field, using the Earthworm Inoculation Unit (EIU) technique (Butt et al. 1995), in two horizontal transects running across the field at 6 m and 8 m distances from the upper field margin (Nuutinen et al. 2006). In each of the four strips

(A–D), 10 units were inoculated in a regular pattern. Field margins and strip grass boundaries were also inoculated. Altogether 40 units were inoculated in the cultivated area and 42 units at the field margins and strip boundaries.

Initial follow-up in 1998 showed that inoculation had been successful only in the strip margins where *L. terrestris* had also started to disperse from the inoculation points (Nuutinen et al. 2006). In a whole field survey done in 2003, *L. terrestris* was still absent from the cultivated parts of the field, except for a few individuals found next to strip boundaries close to inoculation points, while in the strip boundaries the population had started to grow (Nuutinen et al. 2006).

The inoculation inside the field was already deemed a failure, but when the site was revisited at the end of the fallow period in spring 2008, *L. terrestris* middens were abundant at the upslope end of the field. Subsequent sampling in autumn 2009 demonstrated that an abundance gradient of *L. terrestris* from row #1 to row #2 (see Figure 1) had developed inside the field. Abundances in strips B and D were higher than in previously continuously ploughed strips A and C, and also higher above the sub-drain lines than between them (Nuutinen et al. 2011). Subsequent follow-ups have documented the growth of a *L. terrestris* population in the no-till strips and the advancement of a dispersal front towards the downslope end of the field (Nuutinen et al. 2017; Honkanen et al. 2021).

## 2.2 | *L. terrestris* Abundance and Impact on Soil Porosity

### 2.2.1 | Follow-Up Data of *L. terrestris* Density

The present study thus focuses on the two no-till strips B and D (Figure 1). The analyses use data of *L. terrestris* density measured at all four rows (rows #1–#4, Figure 1) in years 2003, 2009, 2013, 2018, and 2022. As the five samplings were done in separate projects, their methodology, sample size, and positioning of samples differed (Table S2). Most notably, in the sampling of 2013, middens of *L. terrestris* on the soil surface, not individuals, were counted (Nuutinen et al. 2017) as midden counting has proved to be a reliable method for *L. terrestris* density estimation under the prevailing conditions (Singh et al. 2015). All field data was converted to density per square meter, and in the statistical analyses, plot means were used. Mass data was not used as it was not available for all time points.

### 2.2.2 | Distribution and Abundance of *L. terrestris* and Their Burrows in 2022

In May 2022, prior to the sowing of spring cereals, the distribution and abundance of *L. terrestris* individuals and burrows were studied across the field. Here the focus was again on the two no-till strips. At the four rows of each strip, measurements were made at two locations situated directly above the plot's two subdrain lines. The sampling pits were positioned as close as possible above the drain lines with the help of the permanently installed poles in the field margins that mark the subdrain positions (Figure S3a). Pits were placed at 5 m distance from plot margins where no previous soil or earthworm sampling had taken place.

At the place of sampling, a 0.6 m × 0.4 m × 0.25 m pit was dug, its longer side aligned with the direction of the subdrain. To expose *L. terrestris* burrows, the bottom of the pit was prepared and levelled, and loose soil removed with a generator-driven vacuum cleaner. The final depth of the pit was approx. 0.3 m. The number of cylindrical macropores which could be reliably recognized as *L. terrestris* burrows by their diameter ( $\geq 5$  mm), vertical orientation and continuity were counted from the exposed surface (Figure S3a). After that, *L. terrestris* were extracted from the pit bottom with AITC application (ISO 2018) and emerging individuals were collected in tap water (Figure S3b). Simultaneously to chemical extraction, *L. terrestris* were hand-sorted into tap water from soil previously excavated from the pit (Figure S3c). In the field, confirmation of live *L. terrestris* identity was based on the unique coloration (clear dorsal pigment gradient from head to tail; Figure S3d) and much larger size of this species compared with other species in the community. Each individual was weighed with a field balance after blotting dry (Figure S3d). The pits were backfilled and earthworms returned into the soil. For statistical analyses, *L. terrestris* total density and mass, and the burrow density, were converted to values per square meter.

## 2.3 | Subdrain Discharge and Precipitation Data

Two subdrain outflow data sets were compiled and utilized to study the (1) long-term impacts of *L. terrestris* population growth on water flow and environmental loads via drain discharge in the strips under no-till, and (2) impacts of *L. terrestris* abundance on hourly discharge dynamics during high rainfall events in the no-till strips.

The long-term data was based on continuous records of subdrain discharge quantities from 2002 to 2022. The variables studied were water volume, total P (acid persulphate digestion in an autoclave; LaChat Quick Chem method 10–115–01-1-F), particulate P (total P less dissolved P), dissolved P (passed through 0.2 μm Nuclepore; LaChat Quick Chem method 10–115–01-1-A), total N (alkaline persulphate digestion in an autoclave; SFS 3031: 1990) and total solids (evaporation residue; SFS 3008: 1990 with minor modifications). For other details of laboratory analyses, see Turtola and Paajanen (1995) or Uusitalo et al. (2024). Phosphorus and nitrogen analyses were performed using a LaChat 8000 QuickChem flow-injection analyzer (Lachat Instruments, Loveland, CO, USA) and, since 2016, using a continuous flow analyser Skalar San++ (Skalar Analytical B.V., Breda, The Netherlands). The instruments were calibrated daily. Analytical performance was monitored by analysing control standards at approximately 20-sample intervals during each analytical run, enabling automatic baseline correction. Newly prepared standards were routinely compared with previously used standards to ensure consistency. Blanks were analysed at the beginning of each run and immediately prior to baseline correction. After each run, all calibration standards were also measured. A calibrated analytical balance was used for all weighings.

In the statistical analyses, three-year means centered at the five *L. terrestris* follow-up years were used (Section 2.2.1), except for the last year (2022), where the mean of 2020–2022 was used. The two-decadal data covered the period when the *L. terrestris* population density increased from negligible to the highest observed densities.

Water discharges were measured continuously with tipping buckets of 4.5-l volume, equipped with magnetic counters connected to an online monitoring system (Netbiter Argos) where the data separation interval is one hour. From the volume of water discharged, a flow-proportional sample (0.1% fraction of the flow) is separated for laboratory analyses of nutrients and sediment matter. For long-term data, the mean temporal interval of the flow-proportional sampling for water chemistry was two weeks (median 5 d, standard deviation 25 d, 828 time-steps). The water, nutrient, and sediment discharge data were first calculated to monthly sums and further to sums and means of the given periods.

For short-term water discharge dynamics, the data used consisted of hourly measurements of the amount of drain discharge during 12 days of exceptionally high rainfall on wet soil. During the selected days precipitation was  $> 16 \text{ mm day}^{-1}$  following at least 10 mm precipitation in the preceding three days. In the local climate this rain intensity is high, with precipitation of  $20 \text{ mm day}^{-1}$  being classified as heavy rainfall by the Finnish Meteorological Institute (FMI). The 12 days chosen came from years 2017 to 2022, a period with high spatial variability in *L. terrestris* density. In addition to the outflow quantities, precipitation time series with the same temporal coverage and resolution were collected from a nearby (600 m distance) meteorological observatory operated by the FMI (Jokioinen Observatory).

## 2.4 | Tracer Element Study

For tracing topsoil movement to subsurface drains, strongly soil-adhering fallout  $^{137}\text{Cs}$  was utilized as an indicator element, as for example, by Laubel et al. (1999). To establish a vertical gradient of  $^{137}\text{Cs}$  in soil, rows #1 and #4 were sampled in 2019 layer-wise with 10 cm increments to 30 cm and then at 30–60 cm depth using a 30-mm diameter soil auger. A laboratory sample was composed of about 20 subsamples. In the laboratory, the soil samples were dried at  $+35^\circ\text{C}$  and crushed to pass a 2-mm sieve.

Eroded sediment was collected from composite water samples of about 30-l volume, gathered from flows during autumn of 2020. The sediment carried with discharge waters was precipitated from water with additions of ferric sulfate solution (10% Fe content; PIX-105, Kemira Chemicals, Helsinki) and polyamin solution (Superfloc, Kemira Chemicals, Helsinki). Both chemicals were added to water while stirring, at rates corresponding to 0.2% of the volume, after which the samples were left standing for 2–3 days. Clear water was then siphoned off and the remaining slurry transferred to foil trays, dried at  $+50^\circ\text{C}$  in a forced oven until it turned to flakes, and was powdered using a mortar and pestle. Sediment yields per drainage pipe ranged between 7 and 30 g of air-dry sediment.

The measurements of  $^{137}\text{Cs}$  activity concentration in soil and sediment samples were conducted at Åbo Akademi University using an Ortec (Oak Ridge, TN, USA) GMX HPGe detector with a 20.7% relative efficiency. The samples were poured into Petri dishes that were glued to the detector end-cap and measured for about 24 h. For calibration and quality control,  $^{152}\text{Eu}$  spiked samples of crushed concrete with known activities were included in the measurements. Calculated mean limits of the  $^{137}\text{Cs}$  activity

concentrations were  $3.8 \text{ Bq kg}^{-1}$  for soil samples and  $10.1 \text{ Bq kg}^{-1}$  for sediments; the higher limits of detection for sediments were due to the smaller sample sizes.

## 2.5 | Statistical Analyses

In the analyses of the long-term data, linear mixed models (LMM) were employed for seven dependent variables (discharge, rain-scaled discharge,  $P_{\text{total}}$ ,  $P_{\text{soluble}}$ ,  $P_{\text{particle}}$ ,  $N_{\text{total}}$ , and sediment). The models included strip (B and D), row (#1–#4), year (2003, 2009, 2013, 2018, and 2022), and the interactions between strip and row with year as fixed effects. For discharge and leaching variables, the mean of three years centered at the five earthworm follow-up years was used (for the last year, mean of 2020–2022). The density of *L. terrestris* was treated as a continuous covariate. Correlations between years within a plot were accounted for using a homogeneous compound symmetry (CS) covariance structure.

In the analyses of the 12 days with high precipitation, the models were again delineated into strips B and D, with row-by-row comparisons of *L. terrestris* abundance. Two plots per row served as replicates, with row treated as a fixed effect in the analysis. In addition, the density of burrows was tested as a covariate.

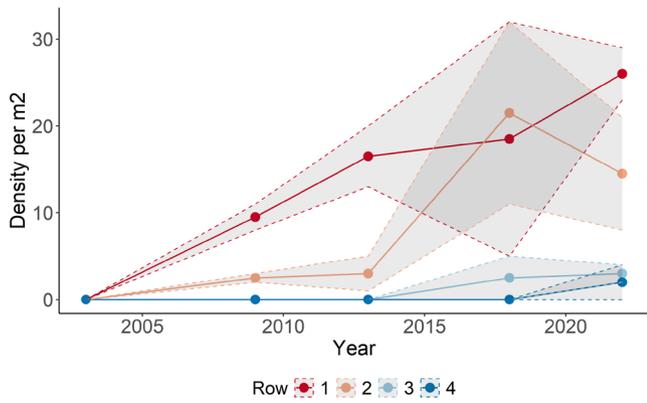
The analysis of *L. terrestris* individual, mass and burrow measurements from 2022 was conducted using row as a fixed effect. Unequal variances for rows were allowed when needed, evaluated using a likelihood ratio test and residual plots. Measurements from the same plot were accounted for by using plot as a random effect.

The models were fitted using the residual maximum likelihood (REML) method, with degrees of freedom calculated using the Kenward-Roger method. Residuals were plotted against fitted values, and the normality of the residuals was found to be adequate in each model. The Tukey–Kramer method was used for all pairwise comparisons of means, with a significance level of 0.05. The R-squared values of the models, which were all high ( $> 90\%$ ), were presented to assess the goodness-of-fit for each model ( $n = 40$ ). All analyses were conducted using the GLIMMIX procedure in the SAS Enterprise Guide 8.3 (SAS Institute Inc., Cary, NC, USA).

## 3 | Results

### 3.1 | Patterns of *L. terrestris* Abundance and Burrow Density

At the start of the *L. terrestris* follow-up in 2003, no individuals were present in the four rows of the two no-till strips (Figure 2). By 2009, population settlement and growth had begun in the two uppermost rows (#1 and #2), with the highest density in row #1 where the inoculation originally took place. The density in row #1 continued to grow over the study period, reaching a maximum of 26 individuals per square meter ( $\text{ind. m}^{-2}$ ) in 2022. In row #2, mean density growth was slower, but in 2018, it was slightly higher than in row #1, before declining somewhat towards the end of the follow-up. In row #3, the first individuals were recorded in 2018,



**FIGURE 2** | The change of *L. terrestris* abundance from year 2003 to 2022 at the two no-till strips of Kotkanoja field from upslope (row #1) to downslope (row #4) plots. Densities are based on individual counts except for 2013 when midden densities were used (see Table S2). Dots indicate the mean, shading the range of values.

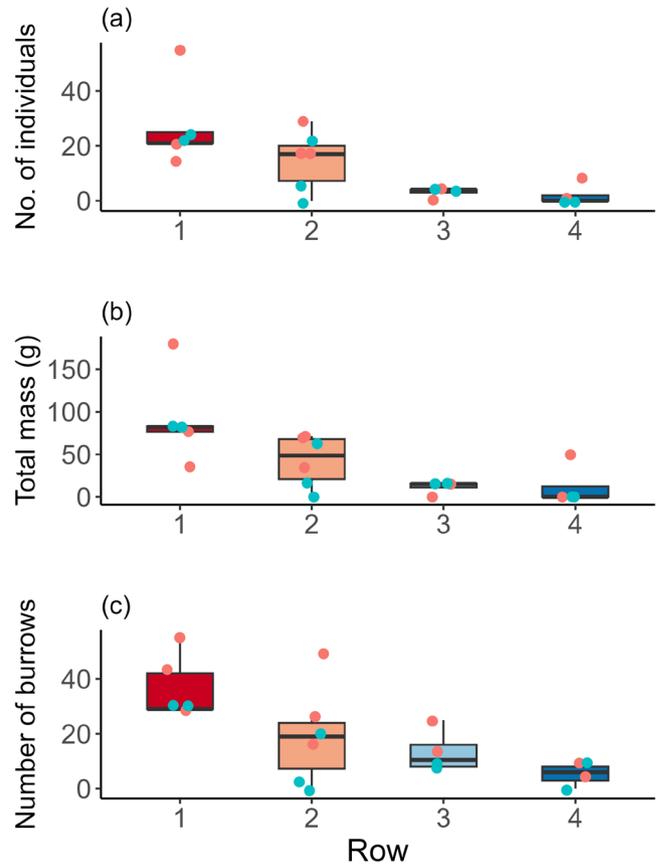
and in row #4, only at the last sampling in 2022. In both of these rows, the mean density remained low, at 2–3 ind. m<sup>-2</sup>.

The results of *L. terrestris* individual and burrow measurements from 2022 are summarized in Figure 3. The downward trend of density from row #1 to row #4 repeats the pattern seen in the long-term data (Figure 3a). The trend for mean mass was similar, with the highest mean mass of 91 g m<sup>-2</sup> (max. 180 g m<sup>-2</sup>) in row #1 (Figure 3b). In row #1, the mean density of *L. terrestris* burrows was 37 m<sup>-2</sup>, with a maximum density of 54 m<sup>-2</sup> (Figure 3c). In row #2, the mean burrow density was 20 m<sup>-2</sup>, and in rows #3 and #4, it was 14 m<sup>-2</sup> and 5 m<sup>-2</sup>, respectively.

In strip D, the one-time plowing to 20 cm was undertaken in July 2018, after which no-till management was resumed. Although the plowing was done in dry midsummer conditions when large *L. terrestris* individuals retreat deeper in the soil thus avoiding direct physical harm caused by the plough, the plowing may have lowered their population density. Namely, looking at the uppermost row #1 with the highest *L. terrestris* densities, the mean density in the following October was in strip D only 5 ind. m<sup>-2</sup> while it was 32 ind. m<sup>-2</sup> in strip B (data not shown). In autumn 2022 the mean densities in strips D and B were 23 and 29 ind. m<sup>-2</sup>, respectively, indicating recovery of the strip D population.

### 3.2 | Long-Term Changes of Subdrain Discharge and Leaching

Drainage flow volumes approximately doubled from the first to the last 3-yr period (Figure 4a). This change was clearly stronger than that of annual precipitation (*cf.* Figure S1), and the discharge-to-precipitation ratio also doubled (Figure 4b). Increases in discharges and discharge-to-precipitation ratio most likely stemmed from variability in within-year precipitation patterns. As for the five periods observed, the first one (years 2002–2004 combined) was characterized by low average precipitation (584 mm year<sup>-1</sup>) and 25% lower than average



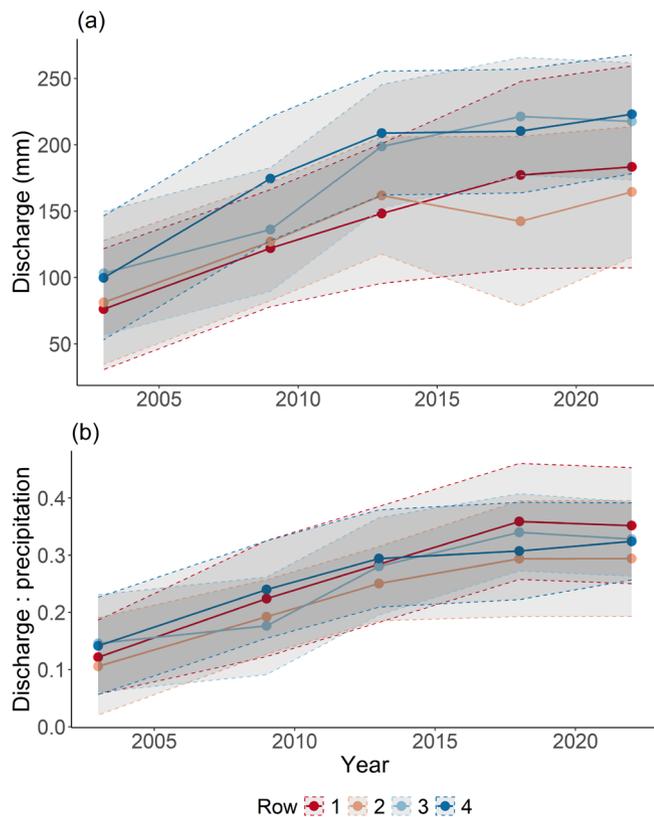
**FIGURE 3** | The density of *L. terrestris* individuals (a), their total mass (b) and the density of *L. terrestris* burrows (c) in May 2022 in the two no-till strips of the experiment (orange dots: Strip B, blue dots: Strip D). All values are given per square meter. Row refers to plot position from upslope (row #1) to downslope (row #4) (see Figure 1). Box-plots were drawn from the raw data (top and bottom of the boxes indicate the 75th and 25th percentiles, respectively, and the line indicates the median). In the plots  $N=2$  and in the rows  $N=4$ , except that  $N=3$  in Strip B rows #1 and #2 and in Strip D row #2. In all cases, evidence was found that row #1 differed from rows #3 and #4 (in (a):  $0.07 < p < 0.09$ ; in (b):  $0.10 < p < 0.11$ , and in (c):  $p < 0.05$ ).

precipitation outside the growing season, the period of a year that is decisive for drainage flow generation. The following four 3-yr periods were characterized by small deviations from average precipitation outside the growing season.

There were no indications for effects of *L. terrestris* density on water discharges, nutrient leaching, and sediment loads as both the row and *L. terrestris* density factors were statistically non-significant for all response variables (Table 1). The interaction row\*year was statistically significant for P forms and sediment, suggesting that these losses were not developing uniformly across all rows. Generally, P and sediment losses were greater from the downslope rows (#3 and #4) than upslope in the first three periods, and in the last two periods the order was mixed (Figures 5a–c and 6b). For water discharge or N, no significant interaction was found, and water discharge was more voluminous from the downslope rows (#3 and #4) than from the upslope rows (#1 and #2) in all periods (Figures 4 and 6a), a pattern opposite to *L. terrestris* density (Figure 2).

### 3.3 | Subdrain Discharge at High Rainfall Events

For the 12 high rainfall events, no statistically significant differences in drainage flow were found between the rows. Figure 7 shows subdrain water volumes for three representative storm events and they exhibit rather random ranking of row discharges from one event to another. Incorporating



**FIGURE 4** | Mean annual subdrain discharge (a) and discharge to precipitation ratio (b) from year 2003 to 2022 at the two no-till strips. Colours mark the rows of plots from upslope (row #1) to downslope (row #4) position. The mean values are model-based estimates of three years centered at the year of *L. terrestris* sampling, except for 2022 for which two preceding years were used. Shadings indicate 95% confidence intervals of the mean.

**TABLE 1** | Statistical significance of fixed effects and their interactions for different response variables in linear mixed models (LMM) of subdrain discharge and leaching in the no-till strips of Kotkanoja field. Significant effects at the probability level of 0.05 are shown bold.  $R^2$ s are shown for each model ( $n = 40$ ).

Factor	Variable						
	Discharge	Rain- scaled discharge	$P_{total}$	$P_{soluble}$	$P_{particle}$	$N_{total}$	Sediment
Row	0.365	0.620	0.301	0.461	0.326	0.9951	0.4623
Year	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>
Row × Year	0.455	0.545	<b>0.008</b>	<b>0.040</b>	<b>0.009</b>	0.419	<b>0.004</b>
Strip	0.416	0.566	0.495	0.059	0.573	<b>0.0254</b>	0.1093
Strip × Year	0.110	0.367	<b>&lt; 0.001</b>				
Density of <i>L. terrestris</i>	0.333	0.521	0.110	0.113	0.209	0.8939	0.1261
R squared	0.915	0.910	0.979	0.945	0.984	0.946	0.986

burrow density in the statistical model did not change the outcome of the analyses, the differences between the rows remaining statistically non-significant (Figure 7).

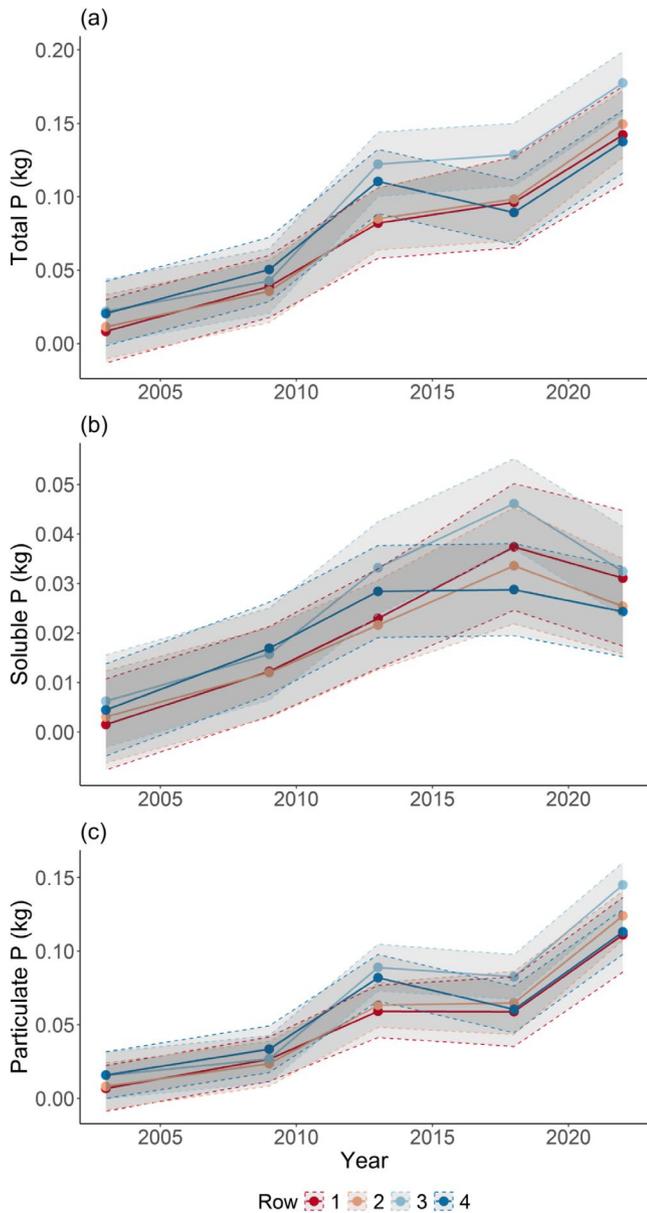
### 3.4 | Topsoil Migration to Drainage Pipes Based on Tracer Element

For strips B and D, the  $^{137}\text{Cs}$  activity concentration in the 0–10 cm and 10–20 cm soil layers was on average  $19.0 \text{ Bq kg}^{-1}$  (range of  $12.4\text{--}21.2 \text{ Bq kg}^{-1}$ ) and  $24.4 \text{ Bq kg}^{-1}$  (range  $18.2\text{--}27.1 \text{ Bq kg}^{-1}$ ), respectively. For both 0–10 and 10–20 cm depths, the samples taken from the upper edge of the field (row #1) had higher  $^{137}\text{Cs}$  activity concentration than the lower end of the field (row #4). In strip B, in which non-inversion tillage practices had been applied since 1991 without interruptions, the  $^{137}\text{Cs}$  activity concentration at 20–30 cm depth dropped to  $6.1\text{--}7.8 \text{ Bq kg}^{-1}$  in both sampled rows. In strip D, where strategic tillage by inversion plowing was made once in 2018, the upper edge (row #1) had an activity concentration below the detection limit of  $3.8 \text{ Bq kg}^{-1}$ , but in the lower edge (row #4) an activity as high as  $21.0 \text{ Bq kg}^{-1}$  was measured. Apparently, the strategic tillage in 2018 had turned soil at the lower edge of the field to a greater depth than the target depth of 20 cm. At 30–60 cm depths, the  $^{137}\text{Cs}$  activity concentrations were below the limit of detection throughout the field.

The activity of  $^{137}\text{Cs}$  in sediment collected from drainage flow was in all cases higher than those measured for the soil samples. Somewhat lower  $^{137}\text{Cs}$  activity concentration was measured in sediment originating from the field's upper end (row #1) than in the lower end (row #4) drains (Table 2).

## 4 | Discussion

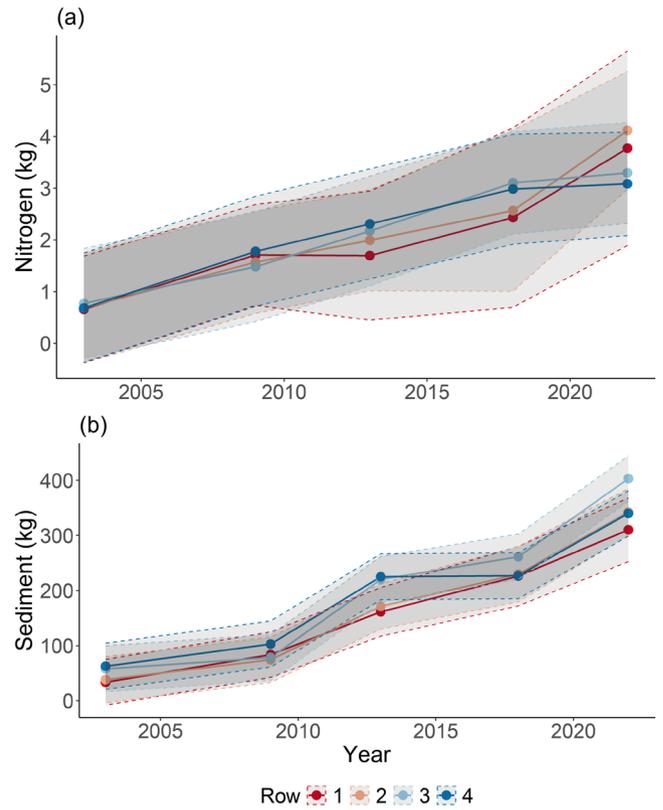
Answers to both of our research questions are negative: (1) in the long-term follow-up, we found no evidence that the gradual increase of *L. terrestris* density had affected subdrain discharge and nutrient losses and (2) the abundance of *L. terrestris* and their burrows was not related to subdrain water discharges during heavy rainfall events.



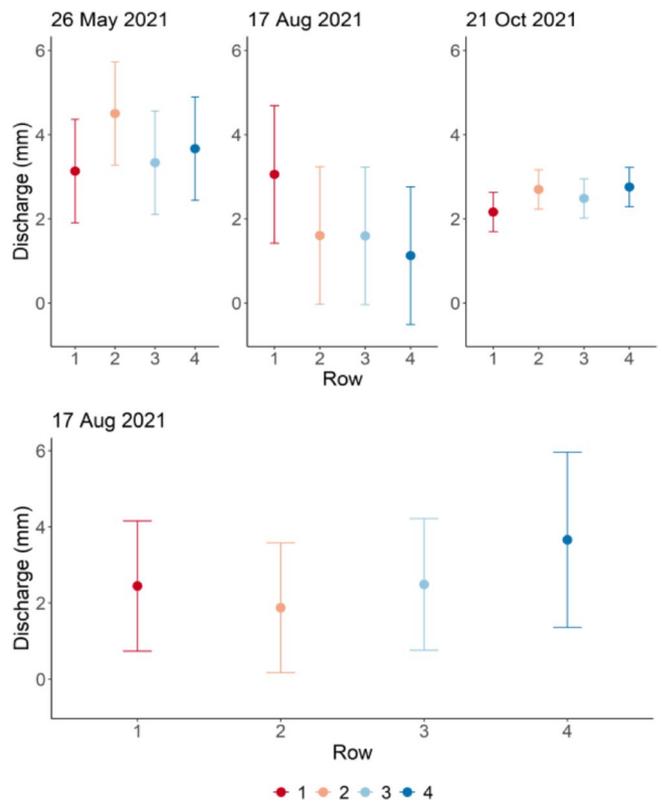
**FIGURE 5** | Mean annual phosphorus load in subdrain discharge: (a) total P load, (b) soluble P load and (c) particulate P load. Presentation and symbols as in Figure 4.

In our earlier studies in long-term cereal no-till fields of S-W Finland, the high mean densities of *L. terrestris* have typically been 20–30 ind. m<sup>-2</sup> (unpublished results) and the highest mean density in the present study (26 ind. m<sup>-2</sup>) corresponds closely with those estimates. This indicates that the gradient of abundance in the field was relevant for the purpose of the study and that the lack of earthworm abundance-related impact on hydrology at the high-abundance areas of the field was not simply because of low *L. terrestris* density. The widening variation of plot measurements towards the end of the follow-up period was likely due to the small physical size of the samples employed in the two last samplings.

The spatially limited impact of *L. terrestris* on soil properties is one possible reason for that no connection between *L. terrestris* abundance and subdrain function emerged. Earthworm follow-up in 2009 showed that the occurrence of *L. terrestris*



**FIGURE 6** | Mean yearly nitrogen (a) and sediment (b) load in subdrain discharge. Presentation and symbols as in Figure 4.



**FIGURE 7** | Subdrain discharge from the four rows in three selected days with high rainfall, based on hourly rainfall data. Top panels: Model-based estimates and 95% CIs without *L. terrestris* burrow density in the model; below: Burrow density added in the model in the case of the 17 Aug 2021 event.

**TABLE 2** |  $^{137}\text{Cs}$ —activity concentration (Bq/kg) in the sediment of subdrain flow at the two no-till strips of the experimental field. Measurements were made from samples collected in autumn 2020.

Row	Strip B	Strip D
1	28.6	38.5
2	33.6	34.0
3	42.5	48.4
4	49.3	43.3
Mean	38.5	41.1

in the field was concentrated at the subdrain lines, the densities being considerably lower already at a four-meter distance from the line (Nuutinen et al. 2011). Thereafter this pattern has remained and is easy to notice before spring sowing due to elevated midden densities and locally exposed soil surface at the subdrain lines due to efficient residue incorporation (Bentley et al. 2024). The potential *L. terrestris* impact on soil therefore mainly involves soil macroporosity and water percolation in the vicinity of the subdrains and in the trench backfill, in the manner earlier described in a nearby clay field by Shipitalo et al. (2004). It is not known how large a proportion of the subdrain flow originates from the percolation above the drain. It is, however, only a fraction of all water entering the subdrains and therefore even a high rate of preferential flow along earthworm burrows may constitute a minor proportion of the total subdrain flow. However, as also noticed by Shipitalo et al. (2004), the hydrological impact of *L. terrestris* can be expanded by soil crack-earthworm burrow interaction: water which enters the soil through topsoil cracks starts to flow laterally in the compact plough-pan and enters subsoil only when encountering *L. terrestris* burrows. Simulation studies have also suggested that lateral flow can be important in the hydrology of clayey fields (Koivusalo et al. 2017).

Direct studies on the contribution of *L. terrestris* burrows to water infiltration under natural field and weather conditions are rare. Edwards et al. (1989) studied the water flow in individual *L. terrestris* burrows in an Ohio no-till corn field during twelve growing season rainstorms with average rainfall of 24 mm in 3.4 h. In the silt loam soil, not all burrows conducted water in a particular storm, but the number of conducting burrows and the flow rate increased linearly with the rain intensity. It was estimated that flow in *L. terrestris* burrows accounted for an average of 4% of total rainfall (range 1%–10%). In a subsequent study which monitored several arable fields under a variety of production practices, the corresponding estimate was < 5% (Shipitalo et al. 1994).

It has been considered that ponded conditions caused by heavy rain would be a precondition for the burrow flow to occur (Shipitalo et al. 2004). In the climate conditions of the present study, the limit for a high rainfall was set at  $\geq 16$  mm per day (24h) which corresponded closely with the local definition for heavy rainfall. With the additional requirement of at least 10 mm precipitation days just before the event, this classification returned a reasonable number of rain events outside the growing season from the database used. It is possible that this rain intensity is too low to cause ponding and trigger significant burrow flow at the well-drained field. This

would be a further explanation for the lack of discernible *L. terrestris* impact on subdrain discharge. The role of burrows may be important in the percolation of water from melting snow, particularly as *L. terrestris* burrows remain open in frozen clay soil (Nuutinen and Butt 2009). Despite snowmelt comprising the main discharge period in Finnish soils, *L. terrestris* occurrence or burrow density appeared, however, not to be linked with the variability of row discharges in our data. Earlier Fukumasu et al. (2024) also showed in laboratory experiments that the degree of preferential flow is not necessarily regulated by macroporosity particularly in near-saturated conditions. Field conditions differ essentially from those of laboratory experiments due to the spatio-temporal variation of multiple environmental factors, including here variation in trench backfill material, making the disentangling of dominating processes difficult. Process-based models could be one approach for discerning the potential impacts of different factors (such as soil hydraulic properties and hydrological conditions) on field scale hydrological data (e.g., Jarvis et al. 2024). Furthermore, it could be beneficial to study the relation of drain discharge generation processes and earthworm burrows in high temporal resolution in locations and conditions where rainfall intensities can be considerably higher than that of the current study.

The concern of particle and nutrient translocation risk caused by *L. terrestris* burrow contacts with subdrains was earlier raised under field management where animal slurries were applied in no-till soils with high *L. terrestris* densities (Shipitalo and Gibbs 2000). In that study, the practice of injecting pressurized slurry into the topsoil likely amplified the flow of liquid via the burrows into the subdrains, and eventually to open ditches and waterways. The conditions differed clearly from those of the present study where chemical fertilizers were band-applied at 3–5 cm depth simultaneously with the direct sowing of spring cereals, using P applications that approximately equaled P offtake by crops. In our study field, no-till management has increased dissolved P losses via drainage discharge as compared to annual ploughing (Uusitalo et al. 2018, 2024), suggesting that a macropore network (extending to soil surface) in the soil is well developed and allows percolation water to bypass P-deficient subsoil that would have the capacity to sorb dissolved P. One-time inversion tillage of the strip D after 10 years of continuous no-till decreased dissolved P (both concentration and loss) by more than 50% (Uusitalo et al. 2024), which shows that dissolved P that migrates to subsurface drains is in this field closely coupled with water percolation pathways from soil surface to drainage depth, and with P stratification of topsoil, more so than general increase in soil P saturation. Nonetheless, we did not find that the density of deep burrowing earthworms would be coupled with higher dissolved P losses within the no-till strips.

As for the origin of the sediment discharging via subsurface drains, the almost doubled  $^{137}\text{Cs}$  activity concentration compared to soil samples points to translocation from the topsoil and the selective erosion of finer, more reactive particles that are able to sorb more  $^{137}\text{Cs}$  than the coarser particles enriched in less reactive minerals such as quartz and feldspars. Enrichment of  $^{137}\text{Cs}$  in sediment matter as compared to topsoil was similar to that earlier reported by Sharpley (1985) for clay and total P in rainfall simulations, and by Stoltenberg and White (1953) for total P in catchment studies. Since *L. terrestris* were abundant

at the upper end of the field and found only in low numbers at the lower end row, the opposite order of  $^{137}\text{Cs}$  activity concentration of drainage sediment suggests that topsoil movement to the subsurface drainage system would not be mainly due to the presence of deep burrowing earthworms. Instead, the different backfill material of tile trenches, or some other characteristic of the field (such as clay and C contents; Klöffel et al. 2024), has affected soil particle migration from topsoil to subsurface drains.

Similarly, sediment and nutrient losses appeared to be uncoupled from the dispersal and row density of *L. terrestris* even when these followed a similar temporal pattern in our long-term data. It is likely that discharges in the field have increased due to the general trends of increasing precipitation and annual mean temperatures in the long-term (Figure S2). The trend for precipitation has become stronger for the period outside the growing season (October–April) and that for temperature during the usually coldest period (January–March) (Figure S2). Together these trends would result in prolonged drainage discharge periods in winter. Also, total water and nutrient discharges via subsurface drains between the study periods were likely affected by the vegetation as evapotranspiration and nutrient utilization by cereal crop stands grown in the later part of this study are known to be less than those of perennial grasses grown during the first 3-yr period (Johnson and Jansson 1991; Turunen et al. 2015).

Epi-anecic earthworms such as *L. terrestris* contribute to arable soil processes in several ways which are in general regarded as beneficial for soil fertility. At our study site the incorporation rate of cereal residues into no-till soil is 1.6 times higher above the subdrain lines than between them because of higher level of *L. terrestris* foraging (Bentley et al. 2024). This activity makes *L. terrestris* burrows rapid conduits for fresh C transport into deep soil horizons as was demonstrated by Don et al. (2008) and relates further to the enhanced nutrient mobilization and availability in the *L. terrestris* burrows noticed by Athmann et al. (2017). The burrows of *L. terrestris* can also promote plant growth in extreme weather conditions by counteracting water-logging under heavy rain (Andriuzzi et al. 2015) and in dry soil conditions by providing roots a direct access to subsoil water (Koch et al. 2021). Together these impacts are likely to contribute to the relatively strong positive impact on crop growth of epi-anecic earthworms (van Groenigen et al. 2014). Based on our results, these favorable contributions of *L. terrestris* activity are not thwarted by elevated subdrain leaching disservice.

## 5 | Conclusions

We found no evidence that the population growth of deep burrowing earthworms would increase subdrain discharge or nutrient leaching in a well-drained boreal no-till clay. This was the case both when long-term change in population density was related with subdrain flow records and when individual, high-intensity rainfall events were studied. As temporal and spatial variation of *L. terrestris* abundance was wide and realistic in our experiment, covering the high and low ends of typical variation, it is unlikely that the lack of effects was due to low levels of *L. terrestris* activity. Our results came from a unique but relatively small experiment under a particular environment

and management and potential environmental risks of preferential flow along deep earthworm burrows would warrant further investigation. The present results nevertheless suggest that the risk of such disservices can remain low.

### Author Contributions

**Visa Nuutinen:** conceptualization, investigation, funding acquisition, writing – original draft, visualization, writing – review and editing, project administration, data curation. **Janne Kaseva:** formal analysis, writing – original draft, writing – review and editing. **Riitta Lemola:** writing – original draft, writing – review and editing, data curation, investigation. **Mika Tähtikarhu:** conceptualization, investigation, writing – original draft, data curation, writing – review and editing, funding acquisition. **Kevin R. Butt:** investigation, writing – review and editing, writing – original draft. **Jan-Olof Lill:** investigation, writing – review and editing, writing – original draft. **Joakim M. K. Slotte:** investigation, writing – review and editing, writing – original draft. **Risto Uusitalo:** conceptualization, investigation, funding acquisition, writing – original draft, writing – review and editing, data curation, formal analysis, visualization.

### Acknowledgments

We thank Ilkka Sarikka and Ari Eskola for their assistance in the field work and Luke's field and laboratory personnel for their many contributions to the collection and compilation of the long-term data sets. Support of Eila Turtola was important for us during the early phases of the study. We thank the LierOja -project steering group members Laura Alakukku and Jari Haimi for useful discussions and two anonymous reviewers for their constructive comments. Open access publishing facilitated by Luonnonvarakeskus, as part of the Wiley - FinELib agreement.

### Funding

This work was supported by Salaojituksen Tukisäätiö sr and Teknillis-Yhteiskunnallinen Tutkimussäätiö sr foundations via LierOja -project.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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### Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** ejss70293-sup-0001-Supinfo.docx.