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Océane Gilibert, Maga Gerino, Dan -Tâm Costa, Sabine Sauvage, Frédéric Julien, et al.. Density Effect of Eisenia sp. Epigeic Earthworms on the Hydraulic Conductivity of Sand Filters for Wastewater Treatment. Water, 2022, 14 (7), 10.3390/w14071048 . hal-03648194

HAL Id: hal-03648194
https://hal.inrae.fr/hal-03648194

Submitted on 21 Apr 2022

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Density Effect of *Eisenia* sp. Epigeic Earthworms on the Hydraulic Conductivity of Sand Filters for Wastewater Treatment

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Abstract: Inside sand filters, as inside other microporous substrates, several invertebrates create temporary burrows that impact on water movement through the filter. Lumbricids *Eisenia fetida* and *Eisenia andrei* live under a wide range of environmental conditions and have a high reproduction rate so they are good candidates for ecological engineering tests. We assessed the impact of these species at different densities (0, 100, 500, 1000 g m^{-2}) on the hydraulic conductivity of small-sized experimental filters made of columns filled with filter sand classically used for sanitation mixed with 5% organic matter. The hydraulic conductivity was recorded every 7 days over 37 days in non-saturated conditions. On day 23, 40 g of peat bedding was added at the column surfaces to simulate a surface clogging organic matter pulse input. Columns with an earthworm density equal or superior to 500 g m^{-2} revealed the highest hydraulic conductivities during the first 21 days. At these densities, the hydraulic conductivity was also restored in less than 7 days after the addition of the surface organic matter, showing the influence of the earthworm species on the resilience capacity of the hydraulic conductivity. It was also highlighted that the hydraulic flow was dependent on the lumbricid densities with an optimal density/effect around 500 g m^{-2} in this specific substrate composition. This study showed that the feeding habits and burrowing activity of both *Eisenia* species significantly enhanced the hydraulic flow in a sandy substrate, providing a sustainable solution to limit the clogging of the substrate similar to the one used in filters to treat wastewater.

Keywords: *Eisenia* sp.; hydraulic conductivity; sand filter; wastewater treatment

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Citation: Gilibert, O.; Gerino, M.; Costa, D.-T.; Sauvage, S.; Julien, F.; Capowiez, Y.; Orange, D. Density Effect of *Eisenia* sp. Epigeic Earthworms on the Hydraulic Conductivity of Sand Filters for Wastewater Treatment. *Water* **2022**, *14*, 1048. https://doi.org/10.3390/w14071048

Academic Editor: Christos S. Akratos

Received: 22 February 2022
Accepted: 22 March 2022
Published: 26 March 2022

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1. Introduction

Substrates of both aquatic sediment and terrestrial soil contain a significant diversity of invertebrates [1]. These invertebrates, by moving through the soil, generate a bioturbation mechanism (i.e., the mixing of soil layers), leading to particles and water fluxes through the substrate [2–5]. Mainly, macroinvertebrates with a body larger than 2 mm are large enough to create macropores and a disruption to the soil horizons, which can lead to a modification of the water flux [6].

Soil macroinvertebrate communities include many insects and other arthropods as well as earthworms [6]. Out of all the macroinvertebrate species present in soil, termites, ants, and earthworms are the three main groups studied [7] because they are considered to be soil engineers due to their important role in several soil processes, including the mineralization of organic matter and microbial activity [8]. Each group has a different impact on the soil physical properties due to different burrowing behaviors, which cause an increase of water infiltration in the soil [9]. The increase of the saturated hydraulic conductivity is positively correlated with the volume of percolating macropores in these burrows, their mean and critical diameters, and their number [10].

Earthworms are soil bioturbating species that correspond with the most important group of soil macrofauna in terms of biomass and are found in most soils around the world [11]. Earthworm species are usually separated into three ecological categories (epigeic, endogenic, and anecic), depending on the type of burrows they make [12,13]. Epigeic species are found in environments that are rich in organic matter and they mainly live between the litter layer and the surface layer of the soil, where they create horizontal burrows in the top 10 cm of the soil [14,15]. Endogenic species mainly create burrows without a preferential orientation with many ramifications mainly between 0 and 20 cm of depth; they rarely come up to the soil surface [7,14]. Anecic species create deep vertical burrows with few ramifications and feed at the soil surface [14].

Eisenia fetida and *Eisenia andrei*, as with other species of their genus, are epigeic lumbricids that are easy to rear and that prefer environments with a high rate of palatable organic matter such as cow manure and compost [16,17]. These species support a large range of environmental conditions (like granulometry), have a short life cycle with a high reproductive rate, and have a high rate of decomposition of organic matter [18]. Epigeic earthworms such as *E. fetida* stimulate the fungal and bacterial activities by means of their predation, the path through their gut, and the making of casts, which improve the overall decomposition of organic matter [18–21]. All these characteristics explain why this species is so frequently used as an engineer species to compost and mineralize organic matter in vermicomposting, vermifiltration or constructed wetland (CW) systems [22–25].

The different burrowing behaviors of earthworms influence the soil hydraulic properties as well as the soil chemical and biological compositions [26–28]. The bioturbation activity of earthworms favors soil macroporosity and water-holding capacity, which increase the related hydraulic conductivity [29]. For example, the presence of *E. fetida* in CW shows potential as a solution to prevent clogging, resulting in improved wastewater pollutant degradation [25,30]. The density of *E. fetida* is a key parameter. If it is too small, then little to no change in the water infiltration velocity will be seen between the filter with or without earthworms [31].

The influence of earthworm bioturbation activity on hydraulic conductivity is of particular interest for sand filters treating sewage water. To date, the sand filter strategies that have been developed are all limited by clogging effects due to organic matter accumulation in the interstitial voids of the macroporous substrates. Among the solutions for improving water infiltration are planted filters (also called CW) made with a sand substrate and hydrophile plants, which are nature-based solutions that mimic the riparian wetlands with their natural vegetation and are located in the floodplain along many rivers. The biological processes supplied by the plant root systems, mostly of *Phragmites* sp. and *Typha* sp., favor water infiltration in a similar way to the rivers banks [32]. Although vertical or horizontal planted filters are also affected by the clogging problem, they can be used as a wastewater primary treatment to filter the main suspended solid matter. Considering on one hand the promising filters of water as a low-cost and sustainable solution and on the other hand the ecological engineering of the earthworms in similar substrates, it is assumed that the addition of earthworms to filters could further improve the infiltration capacity of the filters. The addition of faunal biodiversity such as aquatic and terrestrial oligochaetes as biological engineers could improve the infiltration of water through the substrate, thus favoring the biotransformation of pollutants by an interstitial biofilm when compared with filters that are only sheltering plants as biodiversity.

The SmartCleanGarden concept suggests improving green filter technology to recycle water by means of invertebrate biodiversity involvement inside the filter substrate [33]. The SmartCleanGarden concept is based on the deployment of pilot filters on a university campus as an instrumental network for filter capacity demonstrations. The specificity of this concept is to focus on the biodiversity addition and IoT (Internet of Things) with environmental sensor monitoring to create smart filters.

The water purification performance of a soil depends on its chemical composition, hydrological properties, and biotic components [34]. Many variables enable the characterization of a substrate such as its mineral composition, organic matter content, and porosity, influencing parameters such as the water retention capacity and hydraulic conductivity.

The hydraulic conductivity is defined here and below as the flow of fluids in porous media and transport-related phenomena; therefore, it is considered to be the most important hydraulic indicator when studying how to regulate the flow in a substrate [35]. It is mainly influenced by several soil parameters such as the pore size, organic carbon content, bulk density, and water stable aggregate. It serves as an indicator of the infiltration rate and water retention in soil, which are two important parameters for the filter sand used in sanitation.

The main objective of this study was to assess the influence of the ecological engineers such as earthworms on the physical properties of macroporous substrates in terms of infiltration velocity. The species *E. fetida* was selected because of its large tolerance range towards substrate granulometry and its affinity with organic matter. Although several tests involving this species have previously been performed that showed its interesting influence on the water quality regulation (Appendix A), there remains a prior need to understand its effects on the physical properties of the substrate. This first approach, focusing on the hydraulic parameters, enabled us to explain the effect of the lumbricids on the physico-chemical properties (TSS, COD, TN, etc.) and, therefore, on the water quality. To date, few papers have addressed the link between one earthworm population and its network design with the hydrology of the substrate [25,30,36,37]. These few all preclude the positive effects of these earthworms on the infiltration rates and clogging limitation but demonstrations continue to be required to identify the best conditions to apply to develop experiments with these ecological engineers. The type of species with the appropriate density and substrate to supply the best infiltration rates remains to be emphasized prior to an application to real or pilot-sized filters.

In this study, a rapid literature review of the previous tests carried out with lumbricids in filter conditions led us to the use of a mixture of two species, *Eisenia fetida* and *Eisenia andrei*.

This paper aims to: 1. demonstrate how the *Eisenia* sp. density in the specific experimental conditions (substrate composition, humidity, temperature, etc.) influences the hydraulic conductivity of sand filters; and 2. show how these species are involved in the resilience capacity of the hydraulic properties of these substrates when facing an organic accumulation in the top of the columns. Tests were run with similar filtering sand to the one commonly used in sanitation and wastewater filters (0–4 DTU) for a more direct transfer of these results into the professional field of sanitation applying this nature-based solution. The experimental design was also set to mimic a clogging event with a pulse input of organic matter at the surface of the filter. By doing so, the same experiment enabled us to compare, for the first time, the resilience dynamic of a filter toward a clogging event with and without earthworm effects.

2. Materials and Methods

2.1. Substrate Column Composition

The experiment was conducted in a thermostatic room at 18 °C. Twelve PVC columns (height: 33 cm; Ø 7.4 cm), as illustrated in Figure 1a, were made from top to bottom by: (i) a 23 cm layer of a mixture (with a weight ratio of 95:5) of filter sands following the DTU 64.1 norm (Ø 0–4 mm) obtained from the Vicat quarry at Carbonne in the south of France and peat bedding; (ii) a 3 cm layer of thin gravel (Ø 6–10 mm); and (iii) a 3 cm layer of coarse gravel (Ø 10–20 mm). A geogrid (mesh size = 1 mm) was placed at the bottom end of each column (Figure 1a). Each column contained a total of 60 g of peat bedding at the beginning of the experiment, corresponding with 5% of organic matter of the total substrate (dry weight). This bedding provided a less coarse environment for the earthworms and a source of food, considering that *E. fetida* eat approximately 0.4 g of food g^{-1} per day [22,38].

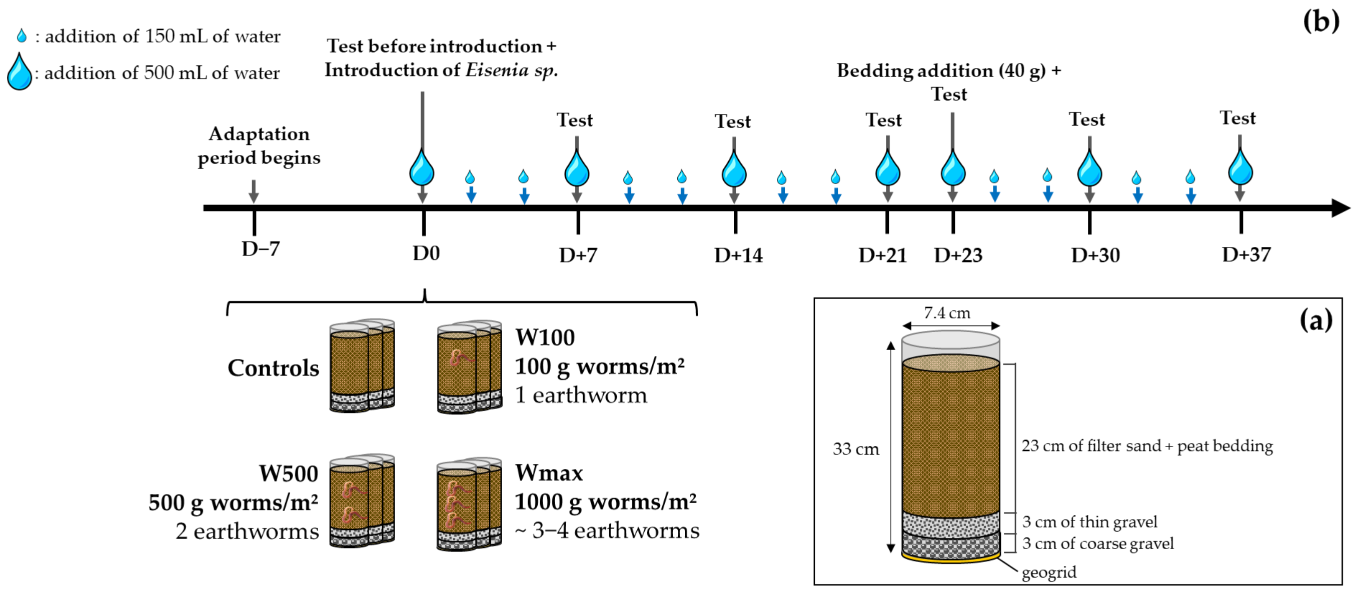

Figure 1. (**a**) Experimental column composition; (**b**) experimental procedure over time, including the hydraulic conductivity test every 7 days (except at D+23) and the wetting of the substrate every 2–3 days.

2.2. Experimental Procedure

Earthworms of the species *E. fetida* were chosen because of their survival over a large range of granulometry; therefore, it was assumed that they could handle the specific substrate currently used in the sand filter. They were bought at the Lumbricid Farm of Moutta located at Boueilh Boueilho Lasque in the south-west of France. However, we were later notified that a few individuals of the *E. andrei* species were present among the population of *E. fetida*. Thus, it was decided to refer to the *Eisenia* genus as *Eisenia* sp. instead of *E. fetida* only as the biological source of bioturbation in this study. *Eisenia* sp. showed a large individual weight variability inside the adult population, ranging from 0.4 g for small individuals to 2 g for larger ones.

Before the beginning of the experiment, 35 g of adult *Eisenia* sp. earthworms were placed into the mix of sand and peat bedding (weight ratio = 95% sand and 5% OM) for 7 days to adapt them to the experimental conditions. The earthworms were then collected by hand by scanning all the substrate they were living in during the adaptation period, before being weighed and introduced into the columns [39]. Three densities of *Eisenia* sp. and a control without earthworms were tested; for each density, three columns were used. The first density, W_{100}, contained 0.4 ± 0.1 g of fresh earthworms per column (corresponding with 1 earthworm per column); that was equivalent to a fresh density of earthworms of 100 g m^{-2}. The density W_{500} contained 2.2 ± 0.1 g of earthworms per column (2 earthworms per column) or 500 g m^{-2} of earthworms. The last density, W_{max}, contained 4.3 ± 0.1 g of earthworms per column (3 to 4 earthworms per column) or 1000 g m^{-2} of earthworms. Note that all earthworms were adults and thus functionally comparable although their individual size and weight differed between the columns. After the introduction of the earthworms, each column was moistened with 150 mL of tap water every 2 or 3 days between the hydraulic tests to maintain an adequate humidity for the earthworms. The experiment lasted for 37 days with repeated measurements of the hydraulic conductivity (every 7 days for the first 21 days). The number of days after the beginning of the experimentation (D0) were expressed as such: D+7 (7 days after the beginning), D+14 (. . .), and D+37 (37 days after the beginning). On D+23, 40 g of peat bedding (0.9 g cm^{-2}) was added to the surface of each column to simulate an input of organic matter at the surface of the DTU sand when used to treat wastewater. After this addition, hydraulic tests were carried out again every 7 days until D+37 (Figure 1b). Three days after the end of the experiment, the columns were disassembled, which allowed us to collect samples of the substrate to measure the moisture and organic matter (OM) content at different depths. Recovered earthworms were counted and weighed.

2.3. Hydraulic Conductivity Assessment

Before each hydraulic conductivity test session, 100 mL of tap water was added to the medium to moisten it. To assess the hydraulic conductivity of each column, 500 mL of tap water was then added to the surface of the substrate. The time needed for this water to disappear from the surface of the substrate was measured as the infiltration time (t_i). This time supplied the hydraulic conductivity (K) equation [40] as follows (Equation (1)):

$$K = \frac{H_S}{t_i} \ln \left(\frac{4V}{\pi D^2 H_s} + 1 \right) \quad (1)$$

where V is the volume of water added, D is the inner diameter of the column, and H_s is the height of the filter sand layer. The impact of the gravel layers on the water infiltration was considered to be insignificant in this case. When the peat bedding was added to the surface of the substrate, the height of this new layer was added to H_s.

2.4. Assessment of Moisture and OM Content

At the end of the experiment, the samples were collected at three different depths in the substrate of each column: in the peat bedding layer (0 cm of depth), in the upper part of the sand layer (10 cm of depth), and in the lower part of the sand layer (20 cm of depth). Three samples per layer were collected. These were used to measure the moisture content with samples placed in the oven at 40 °C for 48 h.

The dried samples were then used to measure the OM content. Two sub-samples were made from each sample of the upper and lower layers (only two samples of bedding layer were produced as they were the same for each column). All samples were set in the oven at 550 °C for 2 h (after 2 h of temperature build-up) to burn the organic matter content in the samples.

2.5. Statistical Analysis

R software was used to analyze all the statistics [41]. Analyses of variance (ANOVA) repeated in time followed by a Tukey test were performed at each date to assess the temporal evolution of the hydraulic conductivity in the cores with different earthworm densities throughout the entire experiment.

ANOVAs were used to test the impact of the density of the earthworms and the depth of the samples on the OM and moisture content in the substrate. As they did not show a distribution normality (Shapiro–Wilk test), the OM and moisture content data were log-transformed to respond to the ANOVA assumptions.

3. Results

3.1. OM and Moisture Content in the Sand Layer

Neither the OM nor the moisture content were different between the earthworm densities (OM: p-value = 0.87; moisture: p-value = 0.97). The sampling depth of the sand layer had a significant impact on the OM and moisture content (OM: p-value < 0.001; moisture: p-value < 0.001; Figure 2). The upper and lower sand layers had a significantly lower OM and moisture content than the bedding layer. In addition, the OM and moisture content were significantly lower in the upper than in the lower sand layer.

3.2. Hydraulic Conductivity

There was no difference of conductivity between the columns before the addition of earthworms (p-value = 0.329) as well as on D+14 (p-value = 0.119; Figure 3). The hydraulic conductivity of the columns before the earthworm introduction averaged $6.47 \times 10^{-5} \pm 0.23 \times 10^{-5}$ cm h^{-1}.

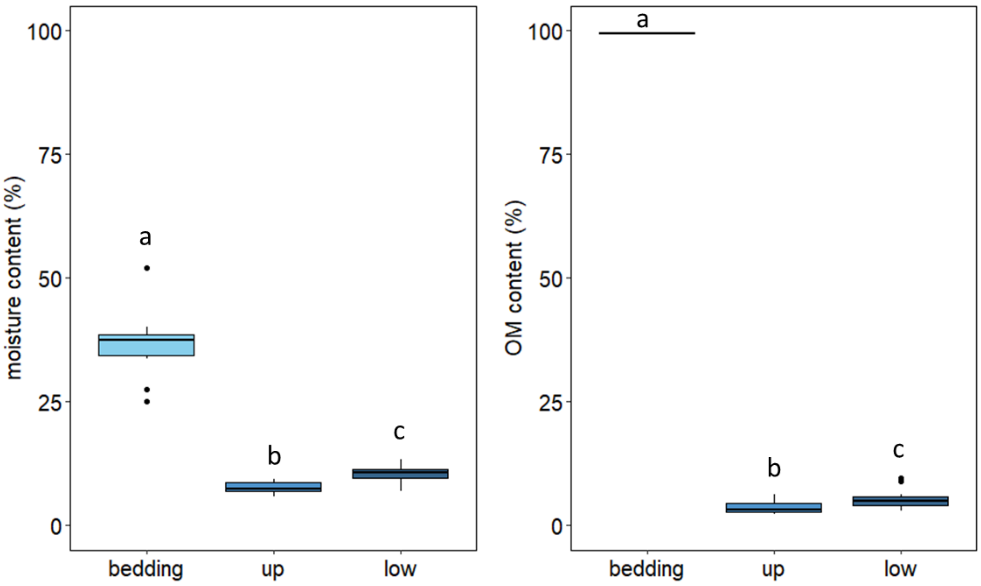

Figure 2. On the **left**: variation of moisture (% of water) at different depths inside all columns. On the **right**: variation of OM content (% organic matter weight in the substrate) at different depths inside all columns. Three depths were used: bedding = in the bedding at the surface; up = upper part of the sand layer; low = lower part of the sand layer. Different letters indicate significant differences between groups ($p < 0.05$). Boxplots represent the median inside interquartile with $n = 36$ (moisture content) and $n = 54$ (OM content).

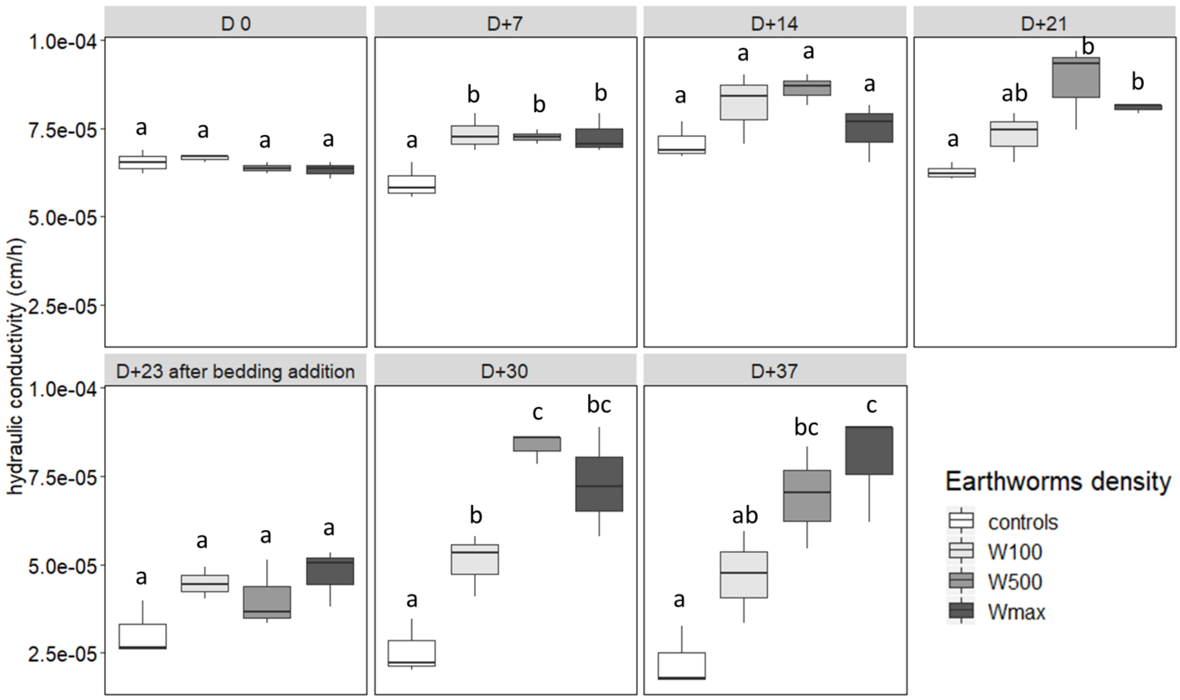

Figure 3. Variations of hydraulic conductivity (cm h^{-1}) for each earthworm density over time (weight of earthworms by column surface: controls = without earthworms; W_{100} = 100 g m^{-2}; W_{500} = 500 g m^{-2}; W_{max} = 1000 g m^{-2}). Different letters indicate significant differences between groups ($p < 0.05$). Boxplots show the median inside interquartile with n total = 84, and n per time = 12.

The hydraulic conductivity of the columns containing earthworms was significantly higher than in the control columns on D+7 (p-value = 0.019). The W_{500} and W_{max} columns had a higher hydraulic conductivity than the controls on D+21 (p-value = 0.012). After the peat bedding addition on D+23, all columns had similar conductivities (p-value = 0.120). Differences in the conductivity were observed on the 30th and 37th day (D+30: p-value < 0.001; D+37: p-value = 0.003). On D+30, all earthworm columns had a higher hydraulic conductivity than the controls and W_{500} had a higher conductivity than W_{100}. Finally, on D+37, W_{500} had a higher conductivity than the controls and W_{max} had a higher conductivity than W_{100} and the controls.

The hydraulic conductivity changed over the experiment time duration for every density (p-value < 0.001; Figure 4). These variations mostly occurred after the peat bedding addition on D+23. The hydraulic conductivity in the control columns was mostly stable over the first 21 days except for D+14; after the peat bedding input, it continuously decreased until the end of the experiment. The W_{100} columns had a hydraulic conductivity less stable than the controls with a spike of conductivity on D+14; contrary to the controls, their conductivity remained low and stable between D+23 and the end of the experiment. In the W_{500} columns, the hydraulic conductivity rose from D+14. It then fell on D+23 but rose again in less than 7 days to equivalent values than those before the peat bedding addition. In the W_{max} columns, the hydraulic conductivity slowly increased during the first 21 days (although this was non-significant). After the peat bedding addition, these columns followed a path similar to W_{500}.

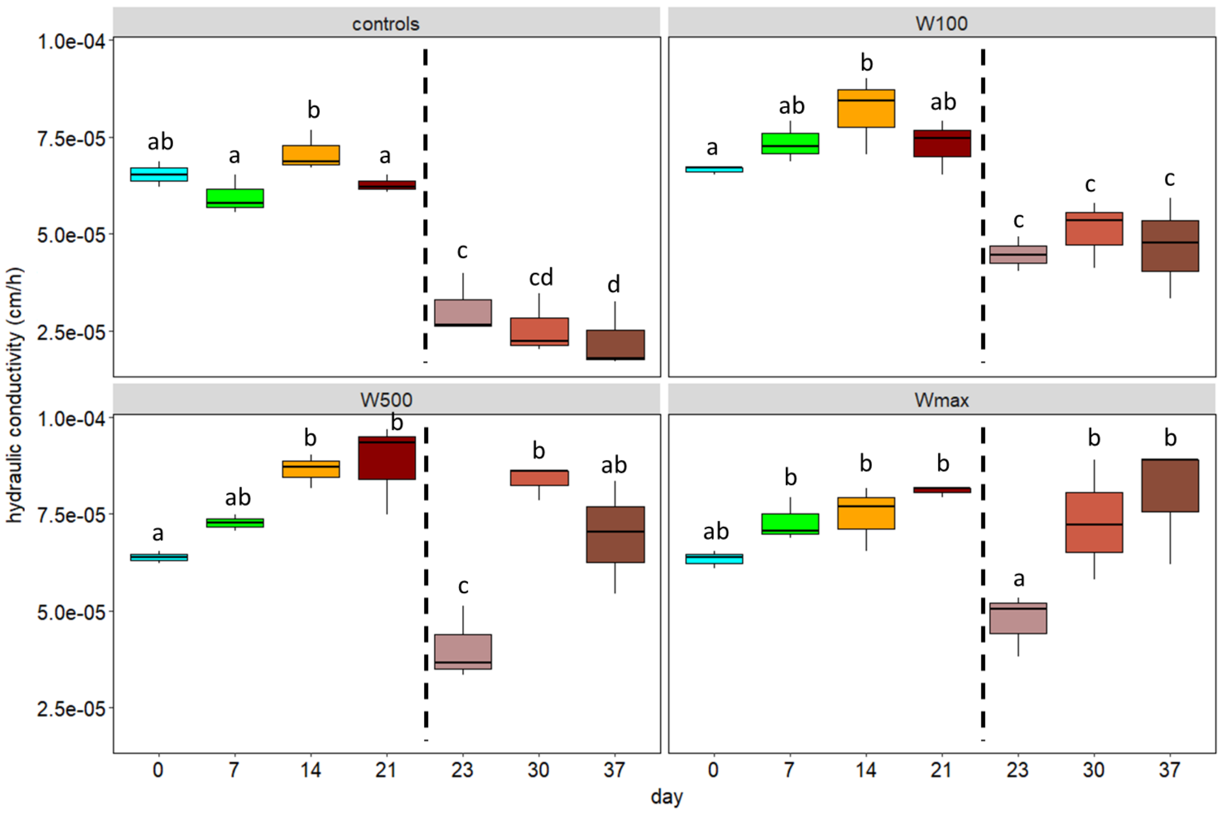

Figure 4. Variation of hydraulic conductivity (cm h^{-1}) over time for each earthworm density (weight of earthworms by column surface: controls = without earthworms; W_{100} = 100 g m^{-2}; W_{500} = 500 g m^{-2}; W_{max} = 1000 g m^{-2}). Different letters indicate significant differences between groups ($p < 0.05$). The pulse input of organic matter at the soil surface occurred on D+23 (just before hydraulic measurement) and is symbolized by a dashed line. Boxplots represent the median inside interquartile with n total = 84, and n per density = 21.

3.3. Earthworm Survival and Biomass Evolution

During the experiment, the earthworm biomass decreased inside the columns with an average decrease of 36 ± 12%. An evasion of earthworms was noticed in the W_{500} and

W_{max} columns, which were found dead next to the columns. Aside from those evasions, the survival inside the columns was 100%. In the W_{500} and W_{max} columns (where there were two or more earthworms), the presence of juveniles was noticed at the end of the experiment during the disassembling of the columns.

4. Discussion

From the literature, sand hydraulic conductivity is typically between 10^{-4} and 10^{-9} cm h^{-1} [42]. A previous study, using the same hydraulic conductivity measurement method as ours, showed that the tested sand (Ø 0–5 mm) had a conductivity of approximately 2 × 10^{-4} cm h^{-1} [43], which was slightly higher than the one measured in our columns before the earthworm addition (6.47 × 10^{-5} cm h^{-1}). However, in a vertical flow constructed wetland (VFCW), a higher conductivity (9 × 10^{-4} cm h^{-1}) was measured before the earthworm addition [36]. This indicated that the conductivity measured in our study was in the same order of magnitude as those from previous studies carried out with the same types of substrates. This validated our method, indicating that sewage filter conditions were properly mimicked in our experiment.

Among the densities tested during this experiment, the W_{500} and W_{max} columns were the only ones for which we observed a significant increase of hydraulic conductivity over time. Significant differences of the conductivity compared with the control columns were only observed after 21 days with the highest conductivity almost equal to 1 × 10^{-4} cm h^{-1} for the W_{500} columns. After the peat bedding addition to simulate an influx of clogging matter at D+23, it took 7 days or less to recover a conductivity similar to that at D+21 (before the peat bedding addition) in these columns. The W_{100} columns did not recover a conductivity similar to before the peat bedding addition but their earthworm density enabled the conductivity to remain constant after the addition of organic material at the top of the column.

The *E. fetida* species is classically used in CW to improve the water flow through the substrate, which becomes clogged over time due to organic matter influx and bacterial biofilm growth [44]. In our study, we worked with two species of *Eisenia* that matched with the in situ conditions of soil colonization.

Our study showed that the hydraulic conductivity inside the sand filters depended on the density of the earthworms introduced. A density of 500 g m^{-2} of earthworms seemed to be the optimal value under our conditions. The W_{max} columns did not show increased values higher than W_{500}, and the W_{100} columns had little to no impact on the hydraulic conductivity. These results confirmed previous ones [25], suggesting that 500 g m^{-2} of earthworms is the best density to restore clogged filters in a limited time (less than 7 days). This density of earthworms also led to an increase with time of the hydraulic conductivity in a VFCW model with different hydraulic loads [36]. Furthermore, a density of 400 g m^{-2} of *Eisenia* sp. was found to be sufficient to triple the effective porosity of the first 20 cm of a sand filter compared with one without earthworms [30]. Therefore, our study results confirmed that the addition of a minimum density of 500 g m^{-2} of earthworms from the genus *Eisenia* enabled the hydraulic conductivity of a filtering microporous medium to be to enhanced. From the current demonstration made with filter sand with a DTU 64.1 (Ø 0–4 mm), which is commonly used for traditional sewage water filters, it was possible to suggest that the addition of a similar density of earthworms in filters made with the same substrate conserved all physical properties but there was an improvement in the hydraulic conductivity supplied by the earthworms.

The addition of OM and suspended solids leads to a reduction of the hydraulic conductivity over time with the reduction speed dependent on the organic load that accumulates in the system [45]. As demonstrated here, the addition of earthworms limited the conductivity decrease of the filtering media by reducing the clogging rate [46]. By their burrowing activity and burrow network setting, the earthworms increased the permeability of the substrate where pores might have been clogged by suspended or dissolved solids, organic matter, or bacterial biofilms [15,47]. If the experiment was transposed into a real sewage sand filter or CW, the bacterial biofilms and detrital OM would likely be consumed by the earthworms, which, together with the buried galleries, would enhance the macroporosity and consequently the hydraulic conductivity and hence diminish the clogging matter [48,49]. Earthworms could also reduce the clogging matter directly by their feeding activity or indirectly by enhancing the bacterial activity that would consume this matter. The sum of these biological activities in the substrate that belongs to natural bioturbation is shown here to favor the resilience of smaller ecosystems, the filter columns. It was assumed that this bioturbation could occur in the same way in larger sewage filters and thus improve their filter functioning.

The water transfer properties of a soil are influenced by the spatial arrangement of the whole burrow system, creating an additional biologically mediated macroporosity in the substrate that superimposes its natural porosity related to particle sizes. This biological macroporosity is dependent of the size, angles, branches, and continuity of macropores biologically produced by the burrows [12,50]. The age and turnover of the gallery networks have also been demonstrated to be important in understanding how they accelerate water flow through the soil [51]. Thus, the type of burrows made by each earthworm functional group should be also considered to predict the influence on the water flux inside the substrate. Earthworms are split into three ecological categories (epigeic, endogenic, and anecic) that can be used as a proxy to describe the burrowing behavior of earthworms, but it is important to keep in mind that bioturbation characteristics can be flexible depending on the environmental conditions so that a few species belong to two ecological categories instead of one [17].

The burrowing activity of the epigeic lumbricid *Eisenia* sp. mainly consists of the generation of horizontal burrows at depths ranging between 0 and 10 cm [15]. Endogenic earthworms also create horizontal burrows [52] similar to epigeic ones but at greater depth (between 10 and 30 cm), which also increases the dispersion and retention time of water inside the soil [53,54]. In addition, compared with their surface counterparts, their burrows are usually less connected to the surface, which limits good water infiltration and lessens the hydraulic conductivity in the substrate [13,55]. Considering the influence of these invertebrate groups on the hydraulic conductivity, anecic earthworms would be the best candidate to achieve an improved conductivity because their vertical burrows are continuous and offer a higher infiltration rate and hydraulic conductivity by linking the bottom to the top of the substrate columns [13,53]. It may also mean that the water residence time is limited, which would be counter-productive for the treatment of wastewater because a longer contact time with the bacteria is required for improving water purification.

Eisenia sp. is usually responsible for a decrease in OM concentrations in any considered system [24,46,56]. In our experiment, at the scale of the columns the density of the earthworms did not influence either the concentration of OM or the moisture content. However, these parameters changed according to the depth inside the column and appeared to be positively correlated, as described in the literature [57]. The OM concentration averaged at 3.6 ± 1.1% in the upper layer of the sand and 5.2 ± 1.8% in the bottom layer. As 5% of bedding was mixed with the filtering sand at the beginning of the experiment, it could be assumed (although not significant when compared with the controls) that the earthworms consumed a part of this bedding in the upper sand layer. This amount of OM (5%) was close to that of the filter bed where the first 10 cm of the substrate contained 5–20% of organic matter as solid particles and then approximately 5% at 10–20 cm of depth [58–60].

Earthworms consumed a small amount of the available peat bedding as their intestines were filled with it at the end of the experiment. However, between the beginning and the end of the experiment, the earthworms lost 36 ± 12% of their initial weight, which was concordant with the threshold of 30% previously set [45]. *E. fetida* earthworms seem to feed on particles with a high C:N ratio of 60:1, which are highly palatable for them [61,62], surplus ammonia could be harmful to them [63]. Generally, peat moss should have a C:N ratio close to 60:1, explaining why it is sold as bedding for *Eisenia* earthworms. The food availability or palatability could, therefore, not be the source of the observed weight loss during this experiment. The substrate moisture content observed three days after the last wetting was lower than the recommended 60% [63]. This deficiency in moisture could have rendered the peat bedding too dry to be easily consumed by the earthworms, which could explain their weight loss. This decrease could also be explained by the evasion of the earthworms from the W_{500} and W_{max} columns because only the total biomass of the worms per column was measured. Aside from this, a few columns of W_{500} and W_{max} (containing 2 to 3 earthworms) had a few juveniles in them; therefore, even if something was limiting the earthworms feeding, it did not prevent them from reproducing in these columns.

The *Eisenia* species was selected in agreement with the literature for its adaptability to a large range of granulometry because this experiment was performed with a substrate similar to the ones used in filters for wastewater treatment. The proportion of organic matter contained in the sand was relatively low compared with the optimal conditions of this genus. The obtained results proved that this species was able to survive in this refractory condition, which appointed it as a as good candidate for further testing in substrates mimicking sand filter compositions.

It is likely that the different OM categories under anthropic effluent additions and an increase in water volume help to reduce the source of stress in real sewage filters. If we use these earthworm populations in filter sand, the specimens should not have any problems adapting themselves to different types of wastewater, either domestic or industrial [8,22,25,36,64,65].

5. Conclusions

The present study was conducted as a preliminary study to pinpoint the potential effect that *Eisenia* sp. could have on the hydraulic patterns of a microfilter column made of DTU filtering sand, which is widely recommended in sanitation systems. The obtained results suggested that the burrowing activity of this Lumbricidae genus with densities superior or equal to 500 g m^{-2} (in our experiment, 2 earthworms/column) could significantly increase the water infiltration in these specific conditions.

Our study demonstrated that an earthworm density of 500 g m^{-2} was optimal to improve hydraulic conductivity and to restore the water transfer properties of an artificial soil back to its initial values in less than seven days after an excess of organic matter was deposited at the soil surface. This observation underlies the ability of these earthworms to participate in the resilience of soil, even in artificial substrates of sand filters and constructed wetlands. In a real-case scenario, *Eisenia* earthworms should mainly live at the surface of the filtering sand where the organic matter accumulates so that they can improve the hydraulic conductivity in the surface layer of the sand. This means that the burrow networks dug by these earthworms should improve the water infiltration rate in the first ten centimeters of the soil. This influence may act in addition to the plant root systems in planted filters and should participate to further reduce the clogging that occurs mainly at this depth. These experimental settings showed that *Eisenia* is a good candidate as a remedy for clogged sand filter using a substrate similar to this experiment by their introduction into existing filters to improve their functioning.

Future investigations need to be carried out to assess the time that earthworms or related functional groups need to adapt themselves in filter sand, and which other earthworm species in combination with *Eisenia* could improve the substrate hydraulic flux in other depth ranges. These combinations may enhance the volume of substrates that is efficient for water treatment.

Author Contributions: Conceptualization, O.G., M.G., Y.C. and D.O.; methodology, O.G., M.G., D.-T.C., S.S., F.J., Y.C. and D.O.; validation, M.G., S.S., Y.C. and D.O.; formal analysis, O.G. and F.J.; investigation, O.G.; data curation, O.G., M.G., S.S., F.J., Y.C. and D.O.; writing—original draft preparation, O.G.; writing—review and editing, O.G., M.G., D.-T.C., S.S., F.J., Y.C. and D.O.; supervision, D.O. and M.G.; funding acquisition, M.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the neOCampus program of the French University Toulouse III–Paul Sabatier.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to acknowledge the neOCampus research device, as well as the SmartCleanGarden concept project (https://smartcleangarden.org/ accessed on 20 March 2022) for the concept support, hydrological advice, and network building. This project received the international research award (Laureate) from the Convergences zero carbon, zero poverty, zero exclusion organization.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A

Table A1. Influence of *Eisenia fetida* density on reduction of chemicals by planted filters vs. controls without earthworms. Percentages written in bold are significant differences and percentages written in italics are non-significant differences. For percentages in brackets, no indication is provided whether the differences are significant or not.

Density (g m^{-2})	Surface (m^{-2})	Type *	Plant	Substrate	Difference In Chemical Reduction between Planted Filters with *E. Fetida* and Controls without Earthworms (%)				
					COD	NH_4^+	TN	TP	TSS
45 [31]	0.18	VPF	*Phragmites australis*	33 cm of sand (2–4 mm); 12 cm of gravel (6–50 mm	*0.0*	/	*+1.0*	*−1.0*	*+1.0*
60 [66]	0.16	HPF	*Lolium perenne*	20 cm of mixed soil; 30 cm of gravel (10–20 mm)	**+5.8**	**+6.2**	**+6.0**	**+9.2**	/
400 [30]	0.25	VPF	*P. australis*	40 cm of peat; 40 cm of sand (0–2 mm); 20 cm of gravel 5–30 mm)	(−1.3)	(+5.1)	/	(+11.7)	(+1.1)
526 [36]	0.71	VPF	*Heliconia rostrate*	75 cm of sand (0.27 mm)	*+5.5*	*0.0*	*0.0*	*−7.0*	*−17.7*
608 [56]	1.125	VPF	*Phragmites communis*	40 cm of coal ash	**+15.7**	**+21.3**	**+20.6**	/	**+11.2**
1000 [25]	0.75	VPF	*P. australis*	1 m of sand (0.5–2.5 mm); 20 cm of gravel (10–30 mm)	(−2.0)	(+5.0)	(+2.0)	(+12.0)	/
1562 [67]	0.12	VPF	*Scirpus tabernaemontani*	35 cm of artificial soil (+ wood chips); 5 cm of gravel (1–5 mm); 10 cm of ceramsite (20–40 mm); 3 cm of coarse gravel (35–45 mm)	**+9.7**	/	*+8.5*	*+7.7*	/
16,000 [8]	0.56	VPF	*P. australis*	50 cm of sand (0–5 mm)	**+8.6**	/	**+6.7**	**+4.5**	/

* VPF: vertical flow planted filter; HPF: horizontal flow planted filter.

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