TRANSACTIONS ON SCIENCE AND TECHNOLOGY

A modified-rhizotron protocol for in-situ observation of earthworm burrowing activity in North Borneo's lowland dipterocarp forests

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ABSTRACT This paper introduced an innovative approach to evaluate burrowing activities of earthworms in the subterranean layer of a lowland dipterocarp forest in Danum Valley, Sabah. The motivation behind this study stemmed from the absence of clear and practical methods of observing earthworms in their natural environment. This study proposed modifications to rhizotron-use; for observing earthworms instead of plant roots, using it as a tool for scientists to monitor and assess their activity underground both qualitatively and quantitatively. This paper includes photographic examples and observation advantages to using the 100 cm x 25 cm belowground terrarium. Although this method has certain limitations, it offers significant insights into their behavior, providing both observational and measurable data. The earthworm Perspex rhizotron holds a considerable promise in advancing annelid research, guiding forest management and could pave way for a more creative data collection in the future.

KEYWORDS: Burrows; Danum Valley; Earthworm; Modified-rhizotron; Sabah
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New Method

INTRODUCTION

Earthworms belong to kingdom Animalia and phylum Annelida; which consists of segmented worms. They are recognized for their distinctive physical traits and burrowing behaviour (Bottinelli *et al.*, 2020). As an invertebrate living underground, they play a crucial role in sustaining and improving soil ecosystems (Hendrix and Bohlen, 2002). Not only making the largest soil pore compared to other soil organisms; they also form temporary or stable burrows that lasts for years which facilitates surface water infiltration into deeper soil layers, even if abandoned (Lee, 1985). These burrows also encouraged plant roots to extend deeper into the soil, enhancing soil aeration and increasing water retention capacity (Yadav *et al.*, 2022). Under controlled conditions, earthworm activity was evaluated by forcibly extracting and placing them into laboratory-prepared PVC microcosm cores or repacked soil columns (Capowiez *et al.*, 2014; Ma *et al.*, 2021), then scanning as well as digitizing them for analysis. Another alternative approach is by adding tracers to rainfall simulations thus making burrows more visible, then excavating the soil profiles to study them (Weiler & Naef, 2003; Schaik *et al.*, 2014). These studies aimed to examine not only the macropores and burrows of earthworms but also their relationship to infiltration. They found anecics and larger macropores rapidly infiltrating water to deeper layers but actual heavy rainstorms leeched all tracer dyes and made soil sections non-excavatable nor observable.

In-situ underground observation of plant roots via the rhizotron have started since the early 1930's by Rogers (1939) as mentioned in Carpenter *et al.* (1985) and stated that most of the data presented at that time were (in their own words) "not useful". However, when technology like ImageJ is utilized (a software constructed by the National Institute of Health) as suggested by Schindelin *et al.*, (2012), processed images can be quantified for statistical analysis; coupling usually inaccessible underground soil sections of the rhizotron to study worm activity. In turn, such data can be used to find relationships; macropores in infiltration studies (Weiler and Naef, 2003) or how it influenced lateral subsurface flow studies through preferential flow (Ehrhardt *et al.*, 2022). Other burrow characteristics can also be measured, like in Capowiez *et al*. (2021) where they estimated mean burrow diameter, its total length, and volume effect on soil bulk density and subsequently, impacts on infiltration. In the context of Sabah rainforests, little is known about its underground worm behaviour. Only a few studies relevant to earthworms have been conducted. In Sepilok, researchers mainly focused on cast production and worm population estimate (Gould *et al.*, 1987). In Danum Valley, they studied cast nitrogen retention and cast tower production (Johnson *et al.*, 2012), while a study in Maliau Basin compared land use changes impact on its population (Rao, 2013).

This study aims to offer new knowledge and to develop a method to measure earthworm activity with the least disturbance possible. By making earthworm activity underground visible and assessable, soil conditions can also be analysed thus, potentially supporting future soil rehabilitation and conservation efforts. This is by modifying the rhizotron, to suit an earthworm investigation. Besides that, burrow mapping obtained through this modified-rhizotron is not simulated nor just modelled, like in mesocosms. Here, both biotic and influencing edaphic factors can be studied simultaneously, behind one transparent Perspex acrylic sheet lodged to an underground soil column. Additional topic of interests can be interweaved in the future, like studying worm cocoons, their hatchlings, or even other soil macrofauna. The scale of future research can also be bigger if there was bigger manpower and purpose, i.e. replication of Perspex installed, its length or depth, increased of observation effort or addition of parameters observed.

METHODOLOGY

Material and Methods

A transparent Perspex acrylic sheet was used per plot and functions as a long-term viewing pane for this study to emulate a rhizotron but for earthworm activity only (refer to 3A Composites (2025) for clarity). For this purpose, it was modified to not include any underground base frame like Green (1992) or Carpenter *et al.* (1985) but instead, be just tightly installed against an excavated soil column and held by two long, L-shaped angle aluminium frame extrusions (40 cm x 5 cm x 1 cm; length x width x thickness). Perspex sheets were cut (longer) based on the following dimension: 100 cm × 25 cm (length x width) with thickness of each sheet no less than 0.4 cm. Not only is it suitable to be carried into the forest; acrylics are lightweight, shatterproof, durable and have great optical clarity (3A Composites, 2025). Plots were initially chosen in a stratified random sampling method. They were at minimum, 50-meters apart but not directly next to any riparian area to prevent the plot's groundwater table easily rising and risking worm burrow destruction. Identifying a target excavation site with signs of earthworm casting activity or confirmed population presence is recommended prior to any digging because natural earthworm distribution is very patchy (clustered) and easily leading to an either high population number or being completely absent (Richard *et al.*, 2012). Each targeted area also avoided obvious, thick tree roots and/or rocky terrain (be it at the surface or subterranean layers) because they prevent the Perspex from lying 100% flat, vertically against the soil column. This also ensures uninterrupted installation works and its whole surface utilized to fully observe earthworm activity.

Soil columns were dug but initial digging works are best done with a shovel, and any further refining of the vertical surface used a sharp machete. Any protruding roots in the soil column were excised while excessive and loose surface litter directly above it was removed (unless naturally glued to the soil surface). This is a precaution to avoid any caving-in of decomposed litter during installation or after, because of rainfall. Perspex was then installed and held in place with aluminium extrusions. If necessary, thick tree branches can be placed diagonally against the extrusions like a brace. According to Green (1992) the viewing pane should block light entering the rhizotron except when taking measurements. Therefore, to prevent all light from reaching the Perspex and since light repels earthworm activity, a black plastic sheet was used to cover its vertical surface. It was held securely by a rock or a tree branch and above it is a custom-made tarpaulin canvas roof placed horizontally - mainly to prevent the Perspex from fogging up. Slightly larger than the dimensions of a modified-rhizotron, a quality canvas is sewed securely onto four polyvinyl chloride (PVC) pipes using braided fishing lines. This roof also prevents damage from fallen branches, leaves or animal disturbances. To avoid attracting wildlife attention, it was covered with some fallen leaves. Excavated soil was then left to naturalize. Modified-rhizotron in this research was observed between July 2018 - December 2022.

Due to this research innovating a rhizotron (originally for slow growing plant roots) experimentally for mobile earthworms, a first observation was conducted a week then a month after installation. However, worm activity was barely observable still; thus, no specific viewing intervals were determined as a surefire timeline for when burrows will appear; only that after each observation, columns are "reset" with the refilling of some sand. Observations were made in sunny or rainy weather. All earthworm burrow presence was traced onto a clear plastic sheet. Plastic sheets were then digitized into the ImageJ software. From these images, burrow total length and total area were measured. Any other observations can also be noted (other soil fauna organism, different burrow types etc.).

There are four major steps in establishing an earthworm modified-rhizotron: site selection, installation, observation, and maintenance. Figure 1 illustrates a frontal sketch example of an underground Perspex earthworm viewing mechanism. Details of its construction is elaborated in sub-subsection installation, below.

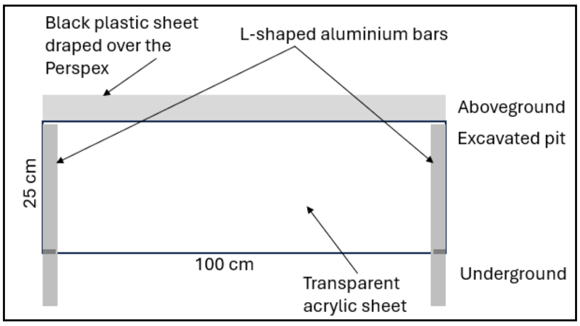

Figure 1. Frontal sketch of an earthworm modified-rhizotron.

Site selection

Perspex rhizotron is easy to set up on a hillslope with less stones or mature roots. Avoid areas known to be human or animal trails and have a history of large mammal species animal tracks such as the Bornean pygmy elephant (*Elephas maximus borneensis*) or bearded pig (*Sus barbatus*). Frequently inundated sites should also be avoided. Sites should be properly marked, preferably by a long, vertical and noticeable PVC pole then site coordinates are recorded using a handheld GPS unit.

Installation

The installation of a modified-rhizotron starts with the careful excavation of a pit up to 25 cm deep and 100 cm long. This field terrarium is up to only 25 cm deep because based on observations in Pérès *et al.* (2010), Capowiez *et al.* (2014) and Capowiez *et al.* (2015), earthworms have the highest activity in the 15 – 20 cm layer. Overall structure, when uncovered and covered is as shown in Figure 2. Removal of the vertical soil surface for the Perspex has to be done carefully to ensure a very straight and even soil surface. It is also aimed at minimising any disturbance to the site because earthworms are very sensitive to vibrations. The L-shaped aluminium bars are buried deep and are used to hold the Perspex sheet closely against the newly excavated soil wall. This research also recommends the use of a thick (> 5 cm) and newly cut tree stem or branch to hold the aluminium bars securely as indicated by arrows in Figure 2(A). A hand-sewn tarpaulined canvas on four PVC pipes were placed above the Perspex rhizotron to avoid its dislodgement and any disturbances as demonstrated in Figure 2(B).

Figure 2. Newly established earthworm modified-rhizotron. (A) A black plastic sheet draped directly above the Perspex. (B) Tarpaulin canvas placed above the rhizotron to prevent damages.

Observation

We highly advised that the earthworm modified-rhizotron is consistently observed by the same individual/s to minimise discrepancies. A scale bar must also be drawn (to scale) onto the transparent sheet used to draw the observed earthworm burrows, specifically during process presented in Figure 3(A). Any earthworm burrows or other differing observations non-earthworm related can be represented by different line widths, dots, or shading (self-defined as long as understood/noted by researcher, to ease calculations when using software) for Results and Discussion. Perspex can be temporarily removed without disturbing the aluminium bars, when verifying drawing accuracy as per Figure 3(B). Photographs should not only be wide angled but also taken up-close. To avoid risk of bias in manual tracings, researchers must learn in advance, how to differentiate a worm burrow from regular soil cracks and gaps. Some examples are given in the Discussion of this study, also in Figure 4(C). Publishing a research's manual tracings and supplementing photographs (or time-lapse imaging) of the Perspex allows a public, inter-observer calibration.

Figure 3. Observation of earthworm underground activities. (A) All on-site observations were recorded by drawing onto a clear plastic sheet. (B) Burrows up-close when Perspex is removed. (C) All gaps and cracks were refilled with sand until compact.

Maintenance

Maintenance of the modified-rhizotron influences the outcome of this study thus was carried out after every observation. The Perspex was firstly removed carefully, washed with water (if available), then wiped with a wet cloth, to remove all residues. Next, it was dried and re-inserted back into the excavated pit. Sand was lightly added from above until no cracks were visible as emphasized in Figure 3(C). Since the soil type in this research were mainly clayish, sand was utilized. However, if soil types of targeted sites were more porous or silty, it is recommended to re-use the same soil available within its plot area.

Assessment of Earthworm Burrowing Activity

The manually drawn earthworm burrow tracings are digitised on ImageJ Version 1.49 software. This burrowing activity can be assessed either qualitatively or quantitatively, once captured as per Figure 4(A) and then mapped, like in Figure 4(B). For instance, the length (cm) of earthworm burrows can be easily quantified while its burrowing patterns (horizontal, vertical, continuity, refilling and collapse of burrows etc.) can be assessed based on data available on the three ecological groups (i.e. epegeic, anecic, and endogeic) from plots. Data collected from their burrowing activity can also be utilized to compare site hydrological properties like infiltration rates and impacts on surface runoff in future research.

Burrows are created when earthworms ingest the soil and for some anecic earthworms, they crush earthworm casts against burrow walls. There are also differences in the way burrows are created as per the visualization presented in Felten and Emmerling (2009). Burrows lengths are also different between endogeics and anecics. Anecic burrows are longer while endogeics burrow numbers are higher though much shorter (Pérès *et al*. (2010); Capowiez *et al.* (2014) and Capowiez *et al.* (2015). All these observable differences may help in discussing earthworm impacts on hydrological processes closely. For example, research by Capowiez *et al.*, 2024 identified a range of burrowing behaviour from earthworm activity in microcosms such as deep and shallow bioturbators, intermediates, barely burrowing litter dwellers and the burrower.

RESULTS AND DISCUSSION

The earliest earthworm burrowing activity was observed a month after the establishment of one field rhizotron, while the others yielded no outcomes and required a longer time for the soil column to naturalize. These burrows were very small, just barely visible against the Perspex wall. There is a methodological limitation here caused by soil column disturbance during installation, which led to the agreed observation time of once soil has stabilized. Earthworm distribution patchiness is dependent on many factors (Edwards, 2004) and some awareness of this can gauge the result success of future studies (e.g. burrowing impossible in dry soil etc.).

In some cases, when inconvenient condensation or mud-smudges appear on its inner wall surface, the first problem can be solved by detaching and cleaning the acrylic panel while the latter is showing signs of earthworm activity. When earthworms are present, digging activities can be ample throughout the viewing terrarium – their features characterized in Figure 4(A) as tunnels surrounded by mud-stains against the clear acrylic, cracks visually lined with an organic or cast material shade which is usually darker than the plot's soil as per Figure 4(C) or continuous, smooth and cylindrical burrows noticeable in Figure 4(B).

To describe Figure 4(B) in detail, bright light-blue markings in the tracings represent large cracks, pockets and gaps in soil, specifically due to physical soil collapse or shifting. However, when these gaps are scrutinized against the digital copy, some of these larger gaps are caused by worm activity because earthworms may have compacted the soil or that it was a feeding or storage area due to traces of organic debris on the burrow walls. Tracing manually on-site requires an immediate decision that can sometimes be affected by the low lighting of a thick canopy forest like Danum Valley, thus, it is best to also double check with a photographed copy. Sometimes, there are random round spaces occurring along an earthworm's burrow (marked in red), which may potentially function as burrow chambers for cocoon deposition, as an earthworm's temporary resting chambers or as a "turning" zones. Navy blue and black lines generally depict worm burrows.

Figure 4. Plot results of an in-situ modified-rhizotron which includes photographs and a manual tracing of earthworm burrowing activity. (A) Measuring burrow length based on photograph using ImageJ software. (B) Tracing of burrows. (C) Visual assessment to verify manual on-site drawings.

As there are differences to earthworm activity outcome in each plot, a pilot study is recommended first – to observe their response because despite worm burrows frequently observed throughout 6 months of Carpenter *et al.* (1985) experiment, this research on the other hand required at least a year of soil left to naturalize, for obvious results. Therefore, tentatively from results presented, the suggested temporal gap between two visits is one year. Preparing many replicates is a strong, precautionary measure against lack of data and confirming worm presence (signs of casting, prior sampling etc.) in the area prior to digging can be advantageous too. Aside from qualitative observation (morphological, spatial distribution or structural analysis of burrows according to ecological group), earthworm burrowing activity also can be quantitatively assessed, in terms of its length, how many openings were created on the soil surface and total burrow volume.

For the plot DV 12B below, only epigeics and anecics are present; sized between 4 – 14 cm, 1 – 5 mm in width but macropores up to 1 – 3 mm only. Within these cracks and gaps, there are different soil shadings similar to casts or humus on burrow walls, suggesting there are anecics and their casts being crushed against the walls as per Capowiez *et al.* (2014) shown in Figure 5(A). Besides that, the burrowing activity is similar to values suggested by Capowiez *et al.* (2024) where its litter dwellers or epigeics have a barycenter starting from 2 cm depth while the epi-anecics (burrowers) can be found up to 6 cm depth and anecics (intense tunnelers) more than 10 cm underground. Other soil macrofaunal activities can also be observed from a modified-rhizotron, for example termites and ants as per Figure 5(B).

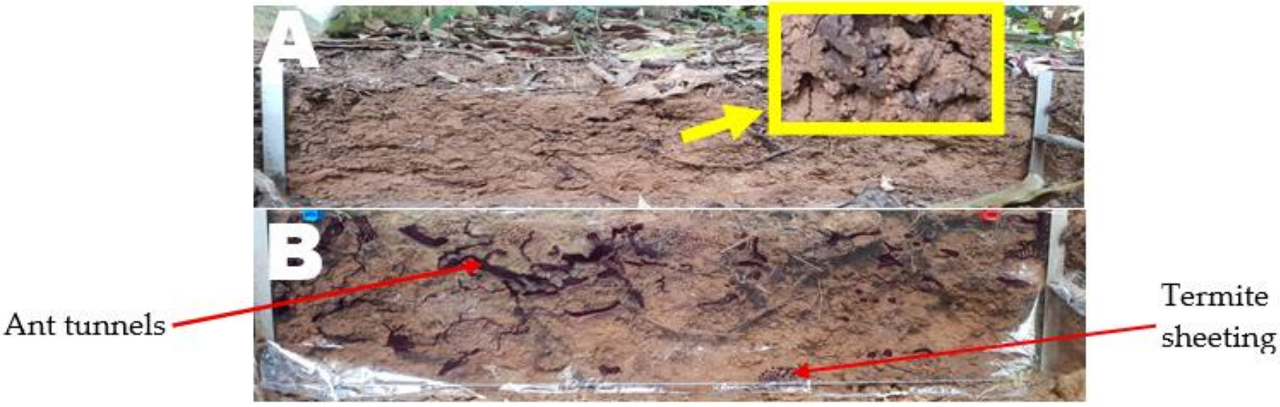

Figure 5. Some plots have multiple soil macrofauna. (A) Earthworm casts found within its own burrow system. (B) Other macrofauna activity depicted by other shadings or markings.

Based on research by Yahya (2025), DV 2 has a dominant number of endogeics, some epigeics and little anecics. According to its underground terrarium set up, no burrows are observable near the surface of the soil column, instead, mainly deep within its soil profile. According to Capowiez *et al.* (2024), endogeics can be separated into different burrowing habits, either a shallow or a deep bioturbator; the first making burrows in the upper soil layers (barycenter 6 cm) while the latter's burrows are deep in the soil (barycenter 9 cm). They both lack surface layer activity, and unpigmented worms are either average sized or small. When paired with information available regarding sampled worms in DV2, they are small to average in size (3 – 7 cm), comprised of both pigmented and non-, with a barycenter of at least 6 cm above and fragmented burrows.

Based on Table 1, the length of burrows in DV 6 is twice as long as compared to DV 12B. One would automatically assume that the infiltration rates for the former being faster than the latter. Earthworm recovered is also half the amount in DV 6. However, based on Table 2, infiltration rates are three times higher in DV 12B and can be explained when a modified-rhizotron is used to compare their burrow structure. Burrows in this plot has many wide pockets of multiple sizes and also larger between 0.5 – 5 mm whereby the ones in DV 6 are fairly uniform in size but only about 0.5 – 2 mm. These measurement comparisons can also be made directly onto the transparent sheet using a digital caliper ruler because the burrow widths are traced exactly like the original observed. Further statistical analysis is recommended to derive a comprehensive discussion and conclusion on this matter.

This study finds manual drawings to be better and more precise than a photographed one, especially once processed in the computer. However, by having a photograph, it allows us to cross-check for potential mistakes. This problem can also be potentially alleviated by a high performance or resolution camera. Findings from using a long term modified-rhizotron may support forest management efforts, if earthworm digging activity is made visible. Their contribution to soil rehabilitation of compacted soil or potential in erosion control is possible once in-situ monitoring and data-driven studies are implemented. From this study, scientists can try to shed light on the significance of worm burrow patterns in dipterocarp forests because there are organic matter and leaf litter found deep within their tunnels; how making nutrients available and improving soil aeration can encourage plant growth and carbon sequestration.

Table 1. Quantitative assessment of earthworm burrowing activity in selected study sites.

Site	Total Length of Earthworm Burrow (cm)		
	First visit	Second visit	Third visit
DV2	120	103	42
DV12B	0	204	887
DV305	62	148	0*
DV6	62	0	1,787
DV17	0	80	0*

*Perspex dislodged on final visit

Table 2. Physical properties, infiltration rates and earthworm information of selected study sites.

Site	Mean bulk density (5 cm; 10 cm)	Elevation (m)	Soil type	Mean resistance (psi)	Site infiltration, mm hr^{-1} (total EW, dominant)
DV2	1.1 ; 1.3	212	Clay loam	157	14 mm hr^{-1} (34, endogeic)
DV12B	1.2 ; 1.4	207	Clay loam > 35%	103	51 (24, epigeic)
DV305	1.0 ; 1.2	216	Clay loam > 35%	116	15 (28, epigeic)
DV6	0.9 ; 1.2	195	Clay loam	133	15 (51, epigeic)
DV17	1.0 ; 1.3	191	Clay loam > 35%	197	15 (53, epigeic & anecic)

The earthworm rhizotron method described in this study offers several benefits and constraints. This method is inexpensive as all essential field equipment are affordable and readily purchasable from photo studios. It provides on-site monitoring of earthworms in their natural setting despite some risks of natural disturbance by wildlife activity, landslides, or flooding. Although earthworm behaviour can be studied qualitatively, and quantitatively, if the rhizotron could be visited at a more regular interval unique results may emerge. One of the biggest limitations of this study is to pinpoint the exact earthworm species creating these burrows presented without causing a disturbance to their population. Therefore, the use of octet electrical extraction in Mazur-Paczka *et al.* (2020) can be considered at the end of the experiment to extract earthworms from field terrariums. Data from the modified-rhizotron can then be analyzed with the species list compiled. Establishing a modified-rhizotron is labour intensive. It also requires adept handling of the machete for accurate and even surface shaving of the soil column. Conversely, maintenance is not frequently required.

CONCLUSION

In conclusion, the innovated method presented in this study can be a practical tool for investigating underground earthworms in the hillslope setting of lowland dipterocarp forests. It can indirectly link earthworms, their burrow properties to its impact on water infiltration in soil aside from potentially observing other soil fauna present during the process. While the Perspex rhizotron has some limitations, it provides valuable insights into earthworm behavior utilizing both qualitative and quantitative parameters. The earthworm modified-rhizotron has the potential to inspire further research on annelids and foster the development of innovative soil management or sampling techniques.

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