Ecosystem engineers: Earthworms in tropical forests: 
A study from Danum Valley, Malaysia

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Abstract
The forest floor of Danum Valley in Sabah, Malaysia, is densely covered with thousands of miniature tower-like soil structures. This study investigated the role of earthworms, which were found to be the creators of these towers, in nutrient retention and soil turnover in tropical forests. Results showed these small earthworms are potentially major ecosystem engineers in tropical forest ecosystems, which turn over and process huge amounts of soil per year (over 88 kg per m²). The worm casts were found to have significantly higher levels of nitrate and conductivity than surrounding soils. It is well known that tropical forests are scarce in nutrients, particularly nitrate. This makes the activity of earthworms potentially crucial for retaining nutrients within the ecosystem and preventing loss through leaching. Soil moisture content was found to be an important factor determining the distribution of earthworms. The implications are especially significant for dry degraded forests where our study showed that the presence of earthworms was lower than in primary and intermediate forests.

INTRODUCTION
It was a muggy October afternoon when we walked through waterlogged forests along the Kinabatangan River in Sabah, Malaysia. Although charismatic mega fauna such as orang-utans, proboscis monkeys and elephants were scarce, the forest floor was studded with thousands of miniature tower-like structures. What were these awkward edifices? And who was the engineer that created them? Living organisms, under the forces of natural selection do not, in general produce structures, using energy, that have no adaptive advantage. If elaborate towers are produced, there must be meaning to the production.

This study investigates the role of earthworms, which we found to be associated with the towers, in nutrient retention and soil turnover in tropical forests. We use the tower-like casts as an indicator for the worm activity. The specific research questions were as follows:

1. What is the distribution of worm casts across different forest types – primary, intermediate, wet degraded and dry degraded?
2. What role do earthworms play in altering the physical and chemical properties of soil, particularly nutrient levels within the overall environment of the forest?
3. What function do the cast structures play for the earthworm?
METHODS

Data collection

Cast density, distribution and soil turnover

Sampling was carried out in four forest types – primary, intermediate, wet degraded and dry degraded. These forest types are defined as:

- Primary forest – undisturbed forest
- Intermediate forest – once logged 18 years previously but still with continuous canopy cover
- Wet degraded forest – clear felled forest with little canopy cover and dominated by pioneer species such as ginger about 3 m tall, located by a stream.
- Dry degraded forest – previously clear felled forest with little canopy cover and dominated by pioneer species such as ginger.

Five 30 m transects were laid out in randomly selected locations – two in the primary forest, one in the intermediate forest, one in dry degraded forest, and one in wet degraded forest.

Two 1 m$^2$ quadrats were set out every 6m along each transect – a total of 10 quadrats per transect. In five of the quadrats, the number of worm casts was counted and the casts collected for analysis. A soil sample was also collected from each quadrat. In the five remaining quadrats, the casts were counted and tagged with wooden toothpicks. These quadrats were re-sampled after five days and the number of new casts counted.

Aeration of soil under worm casts versus soil away from casts

Twenty soil samples of equal volume were collected using a soil corer and tested to see if worm activity changes aeration levels in the soil. The samples were randomly collected across the 4 forest types – 10 from directly under worm casts, and 10 from areas without casts. The degree of aeration per sample was calculated by determining the density (g/cubic cm) – with the assumption that as the density of a volume of soil decreases, the amount of air trapped within the soil increases. The density was determined by first calculating the volume of soil collected in the corer ($\pi r^2 l$) measuring the weight of the soil sample and then dividing the weight by the soil core volume.

Ratio worm:casts

Five 0.25 m$^2$ quadrats containing worm casts were randomly selected in the primary forest. The number of casts was counted and all worms then collected from the top organic layer of soil in each quadrat. (The worms were subsequently separated into morphospecies based on characteristics such as size and shape as well as tegument colour, opacity and bristles; the tube-casts that contained worms
were measured and counted). The worm:cast ratio per quadrat was determined by dividing the number of casts by the number of tube-cast producing worms.

Data analysis

Chemical variables
Twenty-five pairs of cast and soil samples were collected from the m² quadrats on the transects. These were analysed for five variables: pH, microbial activity, nitrate concentration, conductivity and % water content.

The water content and pH values of the soil samples for each m² quadrat were compared with the corresponding total cast weight per m² to test if these variables influenced cast density, worm activity, or distribution of casts.

Water content, pH, microbial activity, nitrate concentration, and conductivities of the soil and cast samples from each m² quadrat were compared against each other to see if worm activity altered the level of any of these variables.

pH
Per soil/cast sample, 50 g of fresh material was mixed with 50 ml of distilled water. A Merck Special Indicator pH indicator strip (range pH 4.0-7.0) was immersed in the soil solution for 1 – 3 seconds, wiped gently and then compared against a pH indicator colour chart after about 30 seconds. The pH readings were then converted to hydrogen ion concentrations for comparison.

Microbial activity/amount of organic material
Microbial activity can be used as an indicator of the amount of labile organic material available within a soil sample, and can be measured by the change in oxygen concentration (mgL⁻¹) over a given time period – since oxygen will be taken up during microbial activity.

Per soil/cast sample: Oxygen uptake was measured using a hand-held Jenway 970 dissolved oxygen meter, calibrated against sodium sulphite solution and saturated air. Fresh sample (50 g) was put into a 500 ml container and the container filled to the brim with water. The oxygen meter probe was then immersed and stirred gently in the water sample until the oxygen concentration reading stabilised. The water sample was then covered with polyethylene to exclude air, and the sample retested approximately every 3 hr over a 24 hr period by which time the oxygen concentration no longer continued to change.
**Percentage water content**

The fresh/wet weight of each of soil and cast sample was noted. The samples were dried in an oven for 24 hr at 100 °C and then re-weighed. The percentage water content of each of the samples was calculated based on the following formula: 100-(100/wet weight*dry weight)

**Nitrate content**

The nitrate concentration of the samples was tested using a CHEMet test method, which uses the cadmium reduction to convert nitrate to nitrite, then diazotisation of the nitrite to give a pink dye, equivalent to the former nitrate concentration.

Per soil/cast sample: 10 g of dried and pulverised soil sample was mixed well with 50 ml of distilled water, and the soil solution then left to rest for at least 30 min to allow any nitrate to dissolve. The solution was subsequently filtered (Whatman standard paper filters), and 15 ml put into a reaction tube. The contents of one cadmium foil pack was added, and the solution shaken vigorously for 3 minutes. The sample was then allowed to stand for 2 minutes. 10 ml of the sample was poured into a sample cup. A CHEMet ampoule was put into the cup and the tip snapped against the side of the cup. The content of the ampoule was mixed by inverting it gently several times. The ampoule was then left for 5 minutes for the colour to develop; the ampoule colour was compared against the CHEMet colour comparator to estimate the nitrate concentration.

**Conductivity**

Conductivity is a measure of mineral/ion content.

Per soil/cast sample: 200 g of dried sample was shaken in a container with 300 ml of distilled water and then left for at least 1 hr for the mineral salts to dissolve in the water, with regular stirring. A conductivity probe was then immersed in the solution and gently stirred until the conductivity reading stabilised.

**Rate of cast and soil turnover**

The following four calculations were made for each forest type, using data per m² quadrat on initial number of casts, number of new casts after 5 days and the weight of fresh worm casts.

- The mean replacement time of a population of casts
- The number of times soil is turned over per year
- The mass of soil turned over (g/yr⁻¹)
- The amount of soil turnover per year
These calculations were made using the following formula. (A logarithmic ‘birth’ rate was assumed in the cast population).

\[ N = \text{number of casts per m}^2 \text{ after } t \text{ days} \]

\[ N_0 = \text{initial number of casts per m}^2 \]

\[ r = \text{birth rate/day}^{-1} \]

\[ N = N_0 e^{rt} \]

\[ r = \frac{1}{t} (\ln N - \ln N_0) \]

Doubling time is a measure of turnover, where \( N = 2 \) and \( N_0 = 1 \)

\[ t^{\text{doubling}} = \frac{1}{r} (\ln 2 - \ln 1) \]

\( t^{\text{doubling}} \) is a measure of replacement time of a population of casts

\( t^{-1} \) is the turnover rate per day.

\[ 365/t = \text{the number of times the soil is turned over per year} \]

\[ 365/t \text{ (mass of soil m}^{-2}) = \text{the mass turned over per year}. \text{This figure can be compared with the mass of soil per m}^2 \text{ to give an estimate of the amount shifted per year}. \]

**Worm habitat**

Soil pits about 0.5 m deep were dug by the transects in the intermediate and wet degraded forest types to examine the profile of the soil. The depth of the top soil was measured and the soil profile examined for the proportion of coarse/fine organic material and to determine where the worms were most active.

**RESULTS**

**Distribution**

**Distribution of worm casts across forest types**

A total of 1,328 worm casts was collected from four different forest types: primary, intermediate, degraded-wet and degraded-dry. The density of worm casts was highest in the wet degraded forest (mean = 130.2 m\(^2\)). In contrast, the density of worm casts was lowest in the dry degraded forest (mean = 2.8 m\(^2\)). The densities of worm casts were significantly different between five pairs of forest types.
Table 1. *P* values from One-way Anova comparing the distribution of worm casts across the different forest types, intermediate, wet-degraded and dry-degraded

<table>
<thead>
<tr>
<th></th>
<th>Degraded wet</th>
<th>Degraded dry</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean density per m²:</strong></td>
<td>41.5 (sd 34.8)</td>
<td>2.8 (sd 2.1)</td>
</tr>
<tr>
<td><em>P</em> value</td>
<td>0.0017</td>
<td>0.0301</td>
</tr>
<tr>
<td>Intermediate</td>
<td>49.6 (sd 21.1)</td>
<td>0.0136</td>
</tr>
<tr>
<td><em>P</em> value</td>
<td>0.0136</td>
<td>0.0011</td>
</tr>
<tr>
<td>Degraded wet</td>
<td>130.2 (sd 53.2)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

However, the difference in density of worm casts was not significant between the primary and intermediate forest (ANOVA, *p* = 0.644) (see Figure 1).

![Fig. 1: Density of Worm Casts across Forest Types](image)

**Figure 1.** Mean density of worm casts in four forest types. Mean values include quadrats with zero numbers.

**Relationship between mass of worm casts and water content in soil**

The relationship between mass of worm casts and soil water content present in samples from the same quadrat was significant (*r* = 0.454; *n* = 25 and 0.025 < *p* < 0.01) (Figure 2). This implies that water content in soil explains 20.62% (*r²* = 0.2062) of the variance in cast occurrence. The regression predicts that worm casts will be absent from soil with less than 17.87% water.
Relationship between density of worm casts and pH of soil
The relationship between the mass of worm casts per square metre was negatively correlated with pH ($r = 0.423$, $n = 22$, $p = 0.025$), with 17.9% ($r^2 = 0.179$) of the variance explained. The mean pH of cast material was 5.2 and ranged from 4.7 to 5.8.

Function of cast building activity in overall soil composition
We investigated the composition of worm casts and soil samples to understand the significance of earthworms and their cast building activity in nutrient cycling.

Comparison of moisture content between worm casts and soil samples
The mean percentage of water was 39.52 in worm casts and 27.47 in soil samples, the difference being significant (ANOVA, $n = 25$, $p = 2.89 \times 10^{-5}$) (Figure 4).
Comparison of conductivity between worm casts and soil samples
The difference in conductivity between worm casts and soil samples was significant (ANOVA, $p = 0.009$). The conductivity of casts in all 8 samples was higher with an average of 86.69 $\mu$s/cm$^{-1}$ in contrast to 64.09 $\mu$s/cm in soil (see Figure 5).

Comparison of nitrate concentration between worm casts and soil samples
The difference between the concentration of nitrate in worm casts and soil samples was significant (ANOVA, $p = 0.0002$). The mean nitrate concentration in casts was 11.79 mg N/kg and 5.59 mgN/kg in soil. Concentration of nitrate in casts was higher than paired soil samples from the same quadrat for all tested samples (Figure 6).
Comparison of pH levels between worm casts and soil samples

We measured the pH levels of worm casts and soil samples and found no significant difference (ANOVA, \( p = 0.08 \)). However, when we compared the concentration of H\(^+\) ions between the two samples, there was a significant difference between worm casts and soil (ANOVA, \( p = 0.048 \)). The concentration of H\(^+\) ions averaged 2.6 x 10\(^5\) M in worm casts and 1.7 x 10\(^5\) M in soil indicating that the activity of building casts makes casts marginally alkaline (see Figure 7 for comparison of pH).

Comparison of soil aeration between worm casts and soil samples

There was no significant difference between soil density of worm casts and soil samples (Mann-Whitney, \( p = 0.186 \)) (Figure 8).

Figure 6. Nitrate concentration in soil v worm casts.

Figure 7. PH of worm casts v soil samples.
Comparison of microbial activity between worm casts and soil samples

We examined the microbial activity by measuring the mean oxygen uptake in worm cast and soil samples. The difference in final oxygen concentrations measured was not significant (Mann-Whitney, $p = 0.5309$). This indicates that the degree of presence of micro-organisms is similar in both casts and soil (Figure 9).

Worm activity – cast and soil turnover rates

Worm activity – measured in cast and soil turnover rates - varied among the forest types (Table 2). Worm activity was highest within the intermediate forest, since by far the greatest mass (88,300 g/year) and proportion (89%) of soil was turned over per year in the intermediate forest; casts
were also replaced at the highest rate. In comparison the results show worm activity is lowest in the dry degraded forest (872 g/year; 0.09%) (see Table 2).

Table 2. Calculations for cast and soil turnover in four forest types. Calculations were made using data per m² quadrat on initial number of casts, number of new casts after 5 days and the weight of fresh worm casts.

<table>
<thead>
<tr>
<th></th>
<th>Replacement time of pop of casts (days)</th>
<th>No. times cast material turned over per year</th>
<th>Mass of soil turned over (g/yr)</th>
<th>Soil turnover per year (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary 1</td>
<td>6.8</td>
<td>54.1</td>
<td>29400</td>
<td>30</td>
</tr>
<tr>
<td>Primary 2</td>
<td>9.7</td>
<td>37.9</td>
<td>23500</td>
<td>24</td>
</tr>
<tr>
<td>Intermediate 1</td>
<td>3.6</td>
<td>103</td>
<td>88300</td>
<td>89</td>
</tr>
<tr>
<td>Degraded – wet</td>
<td>8.9</td>
<td>42</td>
<td>41200</td>
<td>42</td>
</tr>
<tr>
<td>Degraded – dry</td>
<td>11</td>
<td>34</td>
<td>872</td>
<td>0.09</td>
</tr>
</tbody>
</table>

The difference in the mass of soil turned over per year in the primary, intermediate and the wet degraded forest was not statistically significant (Table 2). However the mass of turned over soil was significantly lower in the dry degraded forest than all the other forest types (One way Anova – Primary 1 > P 0.052, Primary 2 > P 0.001, Intermediate > P 0.001, Degraded wet > P 0.001). See Table 3 for details.

Table 3 – P values from One-way Anova comparing the mass of soil turned over (g/yr⁻¹) in the four forest types

<table>
<thead>
<tr>
<th></th>
<th>Primary 1</th>
<th>Primary 2</th>
<th>Intermediate 1</th>
<th>Degraded wet</th>
<th>Degraded dry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary 1</td>
<td>0.807</td>
<td>0.153</td>
<td>0.282</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td>Primary 2</td>
<td></td>
<td>0.100</td>
<td>0.135</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Intermediate 1</td>
<td></td>
<td></td>
<td>0.364</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Degraded wet</td>
<td></td>
<td></td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Earthworm ecology

We measured the ratio between worm casts and worms and found that on average, 1 earthworm is associated with 3.26 casts. We consistently found that casts were more abundant than earthworms for every square metre sampled.

We classified the earthworms into morphospecies and identified the species that is responsible for building the tower-shaped casts that have formed the basis for our study. Species 2 was identified as the cast-maker based on two justifications. Firstly, we observed Species 2 in the casts on three occasions. Secondly, the relative abundance of this morphospecies was higher in soil directly under the casts.

At least 4 different species of earthworms were present in our samples. We also identified at least 3 different types of worm casts. These results are in Table 3.
Table 3. Morphospecies of earthworm collected from soil samples

<table>
<thead>
<tr>
<th>Morphospecies</th>
<th>Description</th>
<th>Total number found in samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species 1</td>
<td>Mean length = 46 mm; mean width = 5 mm, segments are well defined, reddish dorsal side and whitish ventral side; bristles along setae.</td>
<td>3</td>
</tr>
<tr>
<td>Species 2</td>
<td>Mean length = 24 mm; mean width = 2 mm; smooth tegument, no bristles, no ridges along setae.</td>
<td>20</td>
</tr>
<tr>
<td>Species 3</td>
<td>Mean length = 40 mm; width = 1 mm; translucent tegument, no bristles; well-defined ridges between setae.</td>
<td>4</td>
</tr>
<tr>
<td>Species 4</td>
<td>Mean length = 31 mm; mean width = 1.5 mm; double bands on tegument.</td>
<td>1</td>
</tr>
</tbody>
</table>

DISCUSSION

What determines the density and distribution of worm casts?

The forest floor was dense with worm casts in all of the sampled habitats apart from the dry-degraded forest where there were very low numbers. There was a significant relationship between the mass of worm casts per m² and % of water content of the surrounding soil. This suggests that soil water content is an important factor in determining the distribution of worms. Worms were not found in quadrats with less than 17.9%, indicating that worms require a minimum level of moisture in which to live. Soil moisture would not only prevent the worms from dehydrating but also aid movement through the top soil and would be necessary for the intake and digestion of organic matter from the soil. A noticeably high density of worm casts was also observed on the very saturated floodplain soil of Kinabatangan to the east of Danum valley, though the regular influx of fine organic material through flooding may also be a significant factor determining worm abundance there.

The highest density of worm casts was found in the wet degraded forest (mean = 130.2 m⁻²) whilst the lowest number of worm casts was found in the dry degraded forest (mean = 2.8 m⁻²). This suggests that forest quality alone is not a determining factor in worm distribution, but rather a combination of inter-related environmental variables including % soil water content. The regression explained about a quarter of the variance, leaving other factors to explain the remaining three-quarters. Nevertheless, the dry-degraded forest site had the least amount of canopy cover, a factor that would have contributed greatly to the aridity of the sample area since the topsoil would have the most direct exposure to the drying effects of the sun. As such forest quality could indirectly influence worm abundance.

The results also show that pH is a potential influence on the distribution of the worms, because the number of worms decreased as pH increased. This could indicate that the worms are better suited to...
living in more acidic soils – this is in line with worms in temperate forests that are found in higher abundance in acidic soils.

**What function do the worms play in the tropical forest ecosystem?**

Worms are potentially major ecosystem engineers in Danum, which turn over and process large amounts of soil per year through their activity – on average 88.3 kg m\(^2\)/yr (almost 90%) in the intermediate forest. The worm:cast ratio and the number of new casts counted in the monitoring plots over the study period also point towards a high turnover of worm casts, with casts rapidly produced and abandoned. These data correlate with other studies that suggest that the rate of soil turnover by worms in tropical forest ecosystems is possibly greater than that of worms in tropical savannas, shrub or pasture (Gould, 1987). The worms may process such large amounts of soil per year due to the characteristically rapid cycling of organic matter in tropical ecosystems and low availability of nutrient matter in the top soil. Research has also suggested that termites have replaced worms as the main shredders of coarse organic matter and leaf litter and that worms in tropical forests such as Danum mainly process finer organic matter in the top soil itself.

Why is the rate of soil and cast turnover significant? Soils of tropical forest ecosystems are typically old with low amounts of free nutrients. Reasons for this include the prevention of nutrients reaching the forest floor through litter trapping and recycling in the canopy; the rapid recycling of dead organic matter and the rapid take up and storage of any available nutrients. Tropical forest soils are also potentially very leached of minerals/nutrients due to the characteristically high rainfall. In addition, the thin layer of organic top soil in Danum overlays a dense, impervious clay layer with potentially high risks of surface washout of available nutrients. Nutrient retention in the top soils is therefore crucial.

The activity of the worms could potentially play an important role in the recycling and retention of available nutrients. The results show that the casts sampled in all the forest types had higher levels of nitrate and conductivity than surrounding soil. The casts could therefore be an incidental means by which nutrients from fine organic matter in the soil (made available by the worm activity) are retained in the system rather than lost. This nutrient retention could be even more significant in wetter areas which have a higher risk of leaching and runoff. Comparatively higher levels of nutrients in casts than surrounding soils have also been found in studies elsewhere (Mulongoy & Bedoret, 1989, Scheu, 1987).
Why do the worms make the characteristic tubular casts?

The casts have a higher water content than the surrounding soil samples. One possible reason for this difference could be the use of worm secretions to bind the tubular casts together – this was found to be the case in a study on similarly-shaped worm casts (Mulongoy & Bedoret, 1989). If secretions are also used by the worms at Danum to construct their casts, this would suggest that their characteristic structure is not simply incidental to the way they operate but rather an adaptive advantage – the worms would need to invest energy and organic matter to create the secretions and construct the tubes. In addition, regardless of height, the casts were consistently uniform in proportion, shape and structure across all the forest types. Moreover four species of earthworm were identified in the soil samples, however only the one worm species studied in this research was found to make such tubular casts.

The reasons for the construction of the tubular casts are not clear; one hypothesis is the avoidance of predatory soil invertebrates, since the casts were found to be made up of finer particles and harder to break up than soil aggregates. However, very few individuals (4) were found in the casts during the sampling period. Further experimental studies would be needed to determine how and when the casts are made and for what purpose.

CONCLUDING THOUGHTS

In Danum Valley, earthworms are prolific! Our study shows a high rate of soil turnover every year (over 88 kg per square metre) and few earthworms responsible for the bulk of the work. It is well known that tropical forests are scarce in nutrients, particularly nitrate. This makes the activity of earthworms crucial for retaining nutrients within the ecosystem and preventing loss through leaching.

The implications are especially significant for dry degraded forests where our study showed that the presence of earthworms was lower than in primary forests. In such forests, loss of nutrients is augmented during a disturbance (such as damage of canopy cover through logging). The lack of canopy and increased dehydration of soil inhibits the activity of earthworms and could potentially lead to a soil type that is less rich in nitrate and conductivity. Such ecosystems could eventually support lower forest production and plant and animal diversity.

While this study highlights the role of earthworms in nutrient retention and recycling and analyses their preferred habitats, further research is needed to understand the ecology of earthworms. For example, the mystery of why worms build casts remains outstanding. Our pilot studies could be expanded to investigate a higher number of replicates across different forest types and the role of
earthworms in altering the porosity of soil, the potential role of the casts as refuges from predators, and the cycling of nutrients other than nitrogen.

REFERENCES


ACKNOWLEDGEMENTS

We would like to thank the TBA staff for their advice and support during the project, in particular Dr Brian Moss and Dr Jake Snaddon; also the RAs at Danum Valley Field Centre for their help in the field.