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Abstract. 1. Soil infiltration capacity determines the partitioning of precipitation
into infiltration and overland flow and is therefore an important soil hydrological
characteristic. Water infiltration through soil is facilitated by macropores created by
roots and soil macrofauna. In clay-rich soils, such as those of the tropical forests of
Sabah, Malaysian Borneo, most infiltration occurs via these preferential flow pathways.

2. We evaluated the effects of dung beetle tunnelling on infiltration and macropore
creation (depth and width of the flow pathways) in tropical forest soils in Sabah. Using
mesocosms, we applied three treatments (i) soil-only, (ii) dung-only, (iii) dung + dung
beetles, and measured saturated hydraulic conductivity (i.e., the steady-state infiltration
rate) after 0, 5 and 10 days, and assessed depth and width of infiltration pathways by
applying a blue dye tracer.

3. The steady-state infiltration rate increased in the presence of dung beetles, though
differences among treatments were only statistically significant after 10 days. After
5 days of dung beetle presence, infiltrated water had reached a greater depth than the
control mesocosms without beetles. However, there were no differences in the width of
infiltration pathways among treatments.

4. These results reveal the important, but under studied roles of dung beetles on soil
hydrological functioning, that may have consequences for nutrient cycling and plant
productivity. Further, our findings indicate that the novel application of an established
hydrological method–blue dye tracer–can provide interesting and reliable results for
macrofauna–soil interaction studies.

Key words. Ecosystem engineers, infiltration capacity, macropore flow, Malaysian
Borneo, tropical forest soils.

Introduction

Climate change is expected to intensify extreme rainfall events
in the tropics (IPCC, 2013), which will lead to more frequent
soil waterlogging and flooding (Yuan *et al.*, 2005). An increase
in intense rainfall events can lead to greater overland flow and
soil erosion if the rainfall intensity is higher than the infiltration
capacity (Zhao *et al.*, 2018). Increases in overland flow also
lead to a decrease in soil and groundwater recharge, which
can negatively impact soil water availability for vegetation and
streamflow during the dry period (O'Brien *et al.*, 2013).

The infiltration rate of soil depends on soil characteristics,
such as texture, bulk density, soil's organic carbon content, and
root and macropore density (Holden, 2005; Blume *et al.*, 2010).
Many tropical soils are old and highly weathered, resulting
in thick clay-rich soils that have a low matrix infiltration rate
(Hendriks 2010). Infiltration in clay-rich soils is often dominated

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by preferential flow pathways, which are mainly created through biological activity, such as macrofauna or roots (Bachmair *et al.*, 2009; Hassler *et al.*, 2011; Zwartendijk *et al.*, 2017). The occurrence and abundance of macrofauna may, therefore, determine the infiltration capacity and the partitioning of rainfall into infiltration and overland flow. Several studies have focused on the role of roots and organic matter (e.g. Zimmermann *et al.*, 2006; Bachmair *et al.*, 2009; Ghimire *et al.*, 2013), and earthworms or termites (Weiler & Naef, 2004; Jouquet *et al.*, 2012; Fischer *et al.* 2014) on infiltration rates. For example, Jouquet *et al.* (2012) showed that earthworm casts increased infiltration, limiting soil and nutrient losses, whereas termite activity resulted in crusts that increased overland flow and soil detachment. However, other invertebrates, such as ants, spiders, and ground-nesting bees, have also been found to create macropores and increase infiltration rates and can thus aid in soil restoration (Colloff *et al.*, (2010).

Dung beetles are ubiquitous and provide many ecosystem functions and services. They play an important role in bioturbation, secondary seed dispersal, fly population control, nutrient cycling (Nichols *et al.*, 2008) and may also increase infiltration rates (Brown *et al.*, 2010; Forgie *et al.*, 2018). Tunnelling and rolling dung beetle species create tunnels in the soil when nesting or feeding, (Simmons & Ridsdill-Smith, 2011). The size of the dung beetle species influences the diameter of tunnels and tunnelling depth, as well as the dung removal efficiency (i.e., the rate by which dung beetles remove and disperse dung; Halffter & Edmonds, 1982; Nervo *et al.*, 2014; Gregory *et al.*, 2015). By burying dung, dung beetles also increase the amount of organic matter in the soil through bioturbation (Tuma *et al.*, 2019), which has been shown to increase soil aggregate stability and soil water retention (Franzluebbers, 2002). Using an experimental approach, Johnson *et al.* (2016) showed that dung beetle activity may also reduce water stress during dry periods and result in increased plant productivity.

However, there are only a handful of studies that have directly looked at effects of dung beetles on soil infiltration. Using rainfall simulation experiments in South African pastures, Brown *et al.* (2010) found that overland flow was reduced (due to increased water infiltration and soil porosity) but soil losses (amount of soil particles in collected runoff water) were initially higher when dung beetles were present. Similarly, in New Zealand pastures, Forgie *et al.* (2018) observed less overland flow when dung beetles were present, but, in contrast to Brown *et al.* (2010), also less sediment in the runoff for all but the highest simulated rainfall regimes. However, questions remain about the effects of dung beetle tunnelling on infiltration rates in tropical clay-rich forest soils, and whether pathways of infiltration change when dung beetles are present. One study in a deciduous forest and pasture in Vietnam found a positive effect of macrofauna on infiltration. Importantly, this study suggested that dung beetles were particularly relevant and increased infiltration rates 45 times compared to the controls, and had a greater effect than termites (30 times higher) or earthworms (16 times higher; Cheik *et al.*, 2019).

In this study, we further evaluate effects of dung beetles on infiltration in clay-rich tropical forest soil by (i) directly measuring the infiltration rate and (ii) investigating beetle tunnelling behaviour and how it affects the width and depth of preferential flow pathways through the soil using a commonly used hydrological method – blue dye staining (cf. Weiler & Naef, 2004). We expected that through their tunnelling actions, the presence of dung beetles would increase infiltration rate and the depth to which water infiltrates and the average size of infiltration pathways. Furthermore, we investigated whether the effect of dung beetles on infiltration depends on the time since the dung beetles arrived. We assumed that after the initial burial of the dung, the dung beetles would start to nest or create feeding chambers. Therefore, we expected infiltration rate and infiltration depth to increase in the first few days, but to not increase any further after nesting started and tunnelling activity ceased. We therefore hypothesise that (i) dung beetle activity will increase the infiltration rate of tropical forest soils, (ii) the effect of dung beetle activity on infiltration rate will increase in the first few days but will then plateau, (iii) water will infiltrate to greater soil depths when dung beetles are present, and (iv) the average stained path width (i.e., area through which the water infiltrates) will be larger when dung beetles are present. To assess soil infiltration rate and depth of infiltration pathways, we used mesocosms with standardized dung beetle groups. Mesocosms allow some control under realistic environmental conditions, and can bridge the gap between field observations that can generate highly variable results, and laboratory experiments undertaken under nonrealistic conditions (Naeem, 2008).

Material and methods

Study area

This study was conducted in June 2019 in the tropical rainforest surrounding the Sabah Biodiversity Experiment (5°05′18″N 117°38′32″E) in the Ulu-Segama Forest Reserve (Ulu Segama-Malua district) in Sabah, Malaysia. The annual average temperature at the research site is 27 °C and average annual precipitation is approximately 3000 mm year^{-1} (SEARRP, 2019). The forest is classified as a lowland dipterocarp forest, which was selectively logged for economic purposes in the 1980s (Saner *et al.*, 2012). The dominant soil type is orthic acrisol, which developed from bedrock consisting of a mixture of mudstone and sandstone (Hector *et al.*, 2011). The soil has a moderate acidity (pH < 6), is depleted in nutrients (base saturation 81%), and is further characterised by a low organic carbon content at all soil depths, and an increase in clay content with depth (Hector *et al.*, 2011). The study site was located close to the nearest river to facilitate the provision of water for the infiltration experiments (see below) but all sites were located at least 6 m from the river and 2 m above the stream (Figure S1).

Study design

Experimental setup and treatments. Our experimental design consisted of 32 mesocosms that were randomly distributed in the forest (maximum distance 100 m and minimum distance 15 m apart, Figure S1). The area where mesocosms were placed was

Table 1. Number of mesocosms used for each time step (day 1, day 5 and day 10) and treatment (soil-only, dung-only and dung beetles) for infiltration measurements and blue dye experiments.

	Day 1		Day 5		Day 10	
	Infiltration	Blue dye	Infiltration	Blue dye	Infiltration	Blue dye
Soil-only	4	3	4	3	4	0
Dung-only	3	0	4	0	4	0
Dung beetles	5	3	6	3	4	0

a visually similar forest, in terms of vegetation structure and density, and had a similar slope (flat, max 5° slope) and rock cover to ensure comparable conditions for all mesocosms. We did not observe any differences in soil type or texture between the locations of the mesocosms. The mesocosms were plastic rings (diameter 23.5 cm, height 30.0 cm) that were inserted 10 cm into the soil 1 month prior to the start of the experiments to allow the soil to stabilise from the disturbance.

Treatments and time-steps were randomly assigned to each of the 32 mesocosms (Table 1, Figure S1). The three treatments were (1) 'soil-only' (i.e., no dung or dung beetles), (2) 'dung-only' (dung without dung beetles) or 'dung beetles' (dung and dung beetles). We measured infiltration rates in all mesocosms 1 week prior to the start of the experiments (named 'day 0' from here on), and three times afterwards to investigate how dung beetles influence the infiltration rate: after 1 day ('day 1'), 5 days ('day 5') or 10 days ('day 10'). Blue dye experiments were done for the soil-only and dung beetle treatments, but not the dung-only treatments because we were most interested in comparing soil-only and dung beetle treatments. Blue dye experiments were conducted only on days 1 and 5 due to logistical constraints.

Dung collection. We used freshly collected homogenised cow dung from a local farm, as it is commonly used in dung beetle ecosystem experiments (Slade *et al.*, 2011). Individual pats of 700 g were weighed into separate containers and stored in a freezer. Dung was defrosted 12 h before the start of the experiments. Wild cows or banteng (*Bos javanicus lowi*) are still fairly common in the forests of Sabah and around our study site (Journeaux *et al.*, 2018).

Beetle collection. One week prior to the start of the experiments, dung beetles were caught using live collection human dung-baited pitfall traps (Parrett *et al.*, 2019). Human dung was suspended above the trap, with a plate as a rain shield. Leaves were placed in the bottom of the trap and a funnel prevented the escape of the beetles. The beetles were collected after 24 h, sorted by species and sex, and stored in containers with damp tissue paper and some soil until the start of the experiment. Beetles were given liquid from tissues dipped in a cow dung-water solution for 5 days to ensure that they were not satiated when added to the mesocosms and to encourage them to bury and nest. Only tunnelling dung beetles were used in the experiments; rollers were excluded because these species roll their dung balls away instead of burying it underneath the soil pat, and the physical borders of the mesocosm would have altered their natural behaviour.

Dung beetles were selected to represent a community of beetles that have been commonly found in the area (Slade *et al.*, 2007; Slade *et al.*, 2011), as we needed enough individuals for 18 dung beetle mesocosms. Each mesocosm received the same species, number of individuals, and sex (where possible). In total, 25 individuals of seven different species were added to the mesocosms with the dung beetle treatment (see Table 2).

Once the dung and beetles were added, all mesocosms were covered by a fine mesh (1 mm) to avoid dung beetles entering or leaving the mesocosms (Slade *et al.*, 2017). Soil-only and dung-only mesocosms were similarly covered. There were only small rainfall events during the period of the experiments, which could enter the mesocosms. The mesocosms were left for 24 h before day 1 measurements were taken to allow the beetles to utilise the dung. Previous work has shown that dung beetles in Bornean forests will bury a 700 g pat of dung within 24 h (Slade *et al.*, 2011).

Infiltration measurements

Experimental outline. The infiltration rates were measured in all 32 mesocosms 1 week prior to the experiments (day 0) to detect potential differences between mesocosms and treatments due to the high spatial variability in infiltration rates in

Table 2. Overview of the species used in the dung beetle treatments, number of individuals added to each mesocosm, as well as their functional group and body length (mm). We were not able to distinguish the two Catharsius species and therefore treated them as one group. Mean lengths and functional group are taken from Slade *et al.* (2007) and Chiew *et al.* (unpublished data collected in 2018).

Species	Functional group	Body length (mm)	Number of individuals added to mesocosms
O. (*Onthophagus*) *cervicapra* Boucomont	Small diurnal tunneller	6.5	5
O. (*Onthophagus*) *obscurior* Boucomont	Small diurnal tunneller	6.6	4
O. (*Onthophagus*) *mulleri* Lansberge	Small diurnal tunneller	11.0	1
O. (*Onthophagus*) *borneensis* Harold	Small diurnal tunneller	11.0	1
O. (*Onthophagus*) *incisus* Harold	Small diurnal tunneller	9.3	1
Proagoderus watanabei Ochi & Kon	Large diurnal tunneller	14.7	8 (6 females and 2 males)
Catharsius dayacus Lansberge	Large nocturnal tunneller	23.2	5 (3 females and 2 males)
Catharsius renaudpauliani Ochi & Kon	Large nocturnal tunneller	23.4	

Table 3. The average saturated hydraulic conductivity K_{sat} (mm h^{-1}) and 95% confidence intervals (CI) for the different treatments (soil-only, dung-only, and dung beetles) with the number of mesocosms (n) and the P- and F-values of the ANOVA analysis ($K_{sat\_X} \sim$ treatment, day_X). For each time period (day 0 (prior), day 1, day 5 and day 10), we tested if the differences in Ksat among treatments was significant ('after'). Note that negative Ksat values for the lower confidence intervals are physically not possible and were therefore set to 0. Significant ($P < 0.05$) relationships are highlighted in bold (also see Figure S2).

		Day 1		Day 5		Day 10	
		Prior	After	Prior	After	Prior	After
Soil-only	Average	72	125	72	80	72	75
	CI	38–106	23–226	38–106	34–126	38–106	0–172
	n	4	4	4	4	4	4
Dung-only	Average	47	35	114	46	114	98
	CI	0–156	0–136	0–336	2–89	0–336	0–240
	n	3	3	4	4	4	4
Dung beetle	Average	90	164	95	147	30	489
	CI	3–178	105–222	0–200	34–126	0–67	290–688
	n	5	5	6	6	4	4
Statistical analysis	Log-transformed	No	No	Yes	Yes	Yes	No
	F-value	0.66	5.77	0.05	3.04	1.27	23.78
	P-value	0.542	**0.024**	0.951	0.089	0.327	**<0.001**
	Residual d.f.	9	9	11	11	9	9

forest soils (Zimmermann & Elsenbeer, 2008). For soil-only and dung-only treatments, measurements were repeated in the same four mesocosms on day 1, 5 and 10 (see Table 1). For the dung beetle treatment, it was not possible to repeat the measurements in the same mesocosms because flooding of the dung beetle tunnels may kill them. Therefore, measurements were taken at a different mesocosm for each time step (Figure S1). We measured the infiltration rate in the soil-only and dung-only treatments in four mesocosms for each time step. Because of the expected high variability in the infiltration rates for dung beetle treatments, our aim was to take measurements in six mesocosms for each time step. However, several measurements for dung beetle treatments had to be excluded because the tunnels extended beyond the sides of the mesocosm resulting in very rapid infiltration, such that precise and accurate measurements could not be made. Consequently, we obtained five replicates for day 1, six for day 5 and four for day 10 (see Table 1).

Field measurements. The infiltration rate was estimated by measuring the amount of water that needed to be added to maintain a constant water level in the mesocosm over the duration of the experiment. First, we carefully removed all litter, debris, as well as remaining dung if present. For the dung-only treatment, the dung was stored in a plastic bag during the infiltration measurements and replaced into mesocosms after the experiments. Water was slowly poured into the mesocosm to establish a 10-cm ponding depth. We added more water when the water level dropped below the 10-cm water level and each time recorded the volume of water that was added.

The slope of the plot of cumulative amount of added water against time provides an estimate of the infiltration rate. For comparability of the measurements in different mesocosms and days, and to minimize the effect of differences in antecedent soil moisture conditions (due to potential rainfall between days), we determined the infiltration rate during saturated conditions (i.e., saturated hydraulic conductivity; K_{sat}) based on the steady-state infiltration rate. Preliminary studies showed that after approximately 15 min, the point of saturation was usually reached. We, therefore, calculated the saturated hydraulic conductivity based on the average of the five measurements after this time. The infiltration rate was converted to depth per time (mm h^{-1}) by dividing the volume of water that was added by the area of the mesocosm and the time passed.

Data analysis. The influence of treatment on the steady-state infiltration rate (K_{sat}) was tested using linear models. We ran a separate model for each time step (day 0, day 1, day 5 and day 10) to test for significant differences among treatments. We ensured that model assumptions (homoscedasticity, normal distribution of residuals) were met by log-transforming the data when necessary (see in Table 3 where log-transformation was applied). To assess statistical differences among treatments, we used the least significant differences error bars, and 95% confidence intervals (CIs; Hector, 2015). All analyses were conducted in R studio (version 1.0.153), and the *ggplot2* package was used to plot the data (Wickham, 2016; R Core Team, 2019).

Blue dye experiments

Study design. To obtain an understanding of changes in infiltration pathways through dung beetle tunnelling activity, we added a blue dye to the water that was poured into the mesocosms for the infiltration measurements. Afterwards, we dug a soil pit below the mesocosms to determine flow pathways. More specifically, we assessed the maximum depth of infiltration and the average stained path width. Blue dye staining

experiments are a common method in soil hydrology to determine flow pathways and the importance of preferential flow pathways (Flury *et al.*, 1994; Weiler & Flühler, 2004; Trancón & Bogner, 2012).

Blue dye experiments were done for the soil-only and dung beetle mesocosms on day 1 and day 5 (we could not repeat the measurements on day 10 due to logistical constraints). Because a blue dye experiment is a destructive method, we could not apply blue dye to the soil-only mesocosms where we did the repeated infiltration measurements. We, therefore, assigned three soil-only mesocosms for the blue experiments on day 1 and another three for day 5 (see Table 1, Figure S1). In these mesocosms, we added water containing blue dye, allowing it to infiltrate, but we did not measure the infiltration rate. For the dung beetle mesocosms, we directly added blue dye to the water that was used to measure the infiltration rate in each of the six mesocosms that were assigned to measurements on day 1 and day 5. However, only half of the photographs for the dung beetle mesocosm could be analysed because of problems with the photographs (e.g., unfavourable light conditions causing shadows), resulting in a total of three replicates of the dung beetle treatment for day 1 and day 5 (Table 1).

Field measurements. Brilliant Blue FCF dye (Flury & Flühler, 1994, 1995) was added to the water at a concentration of $4 \, g \, L^{-1}$. After the end of the infiltration experiments, the remaining dye was left to infiltrate. Directly after, the mesocosm was removed carefully to avoid disturbing the soil, and a soil pit was dug through the middle of the mesocosm. The depth of the pit extended below the deepest evidence of blue dye (generally 30–50 cm below the surface). We marked the area of the soil profile where blue dye occurred and measured the length and width of the marked area (Fig. 1a,d). We then took photographs of the soil profile with a Nikon D3000 camera. We shielded the soil profile with an umbrella from the sun to obtain even light conditions (the mesocosm were located under a forest canopy) and used a torch to obtain sufficient light for the dye-stained area.

Data analysis. The photographs were geo-rectified and scaled based on the markers to account for the distortion caused by the angle at which the pictures were taken (Fig. 1b,e). Next, the hue range of the blue dye pixels was determined (usually between 160° and 180° on the Hue Saturation Value colour scale, but adjustments had to be made for each individual photograph), so that the images could be transferred into a black (blue pixel) and white (no blue pixel) picture (Fig. 1c,f). This colour-coded image was converted into a matrix where each pixel in the photograph is one cell, with a value of 1 when the dye was detected (black pixel) and 0 when no dye (i.e., no blue hue) was detected (white pixel).

We compared black and white images for different treatments and time steps visually and quantitatively. First, we calculated the percentage of blue dye-stained pixels per millimetre soil depth by dividing the number of black pixels by the total number of pixels per matrix-row (which represented a mm soil depth). Second, we investigated the horizontal length of the blue-stained areas (stained path width, representing the width of the infiltration pathways) per soil depth, using three width categories: 0–20 mm, 20–200 mm, 200–1000 mm (cf. Weiler, 2001). We then determined relative occurrence of the stained width categories and used this to determine the flow type at each depth following the classification of Weiler and Flühler (2004). This classification allowed us to obtain information about the flow regime, and thus, about the interaction between macropores and the soil matrix. The values of both the percentage of blue dye-stained pixels and the relative occurrence of stained path width categories were averaged per 10 mm soil depth to reduce the noise. The data were plotted using the *ggplot2* package in R (Wickham, 2016).

Results

Infiltration rate

The mean K_{sat} on day 0 was 82 mm h^{-1} (median: 48 mm h^{-1}, range: 9–317 mm h^{-1}). There was no significant difference in the mean K_{sat} values across mesocosms for the different treatments before they were applied (Figure S2, Table 3). There was a small but significant effect of the dung beetle treatment on K_{sat} after 1 day and a larger effect after 10 days (Table 3). On day 1, the K_{sat} of the dung beetle treatment appears to differ from that of the dung-only treatment. We consider this small, but significant difference in the K_{sat} values an artefact due to the small sample size. The difference in the K_{sat} of the dung beetle treatment was much larger on day 10 compared to both the soil-only and dung-only treatments (Fig. 2, Table 3). The average K_{sat} of the dung beetle treatment on day 10 was 20 times higher than the average K_{sat} of the same mesocosms on day 0, and 5 to 6 times higher than the average K_{sat} of the soil-only and dung-only mesocosms measured on this day.

Infiltration pathways

The visual inspection of the percentage of stained pixels as a function of soil depth suggests a comparable percentage of blue dye-stained pixels at all soil depths for the soil-only treatment on days 1 and 5, as well as the dung beetle treatment on day 1. These dye results suggest that water percolated to a depth of around 200 mm (Fig. 3). The number of stained pixels rapidly decreased with increasing depth, and maximum infiltration depth was generally between 300 and 400 mm below the soil surface. For the dung beetle treatment on day 5, we observed a comparable percentage of blue dye-stained pixels until a depth of 150 mm, but below this depth percentage of blue dye-stained pixels was generally higher than for the soil-only treatment on day 1 and day 5, and dung beetle treatments on day 1. For example, between 200 and 400 mm depth, more than half of the pixels were stained for two of the three mesocosms compared to only around 0–25% of the pixels in the other treatments (Fig. 3 and Figure S3). Below 400 mm soil depth, the number of blue dye-stained pixels was very small, regardless of the treatment and time point.
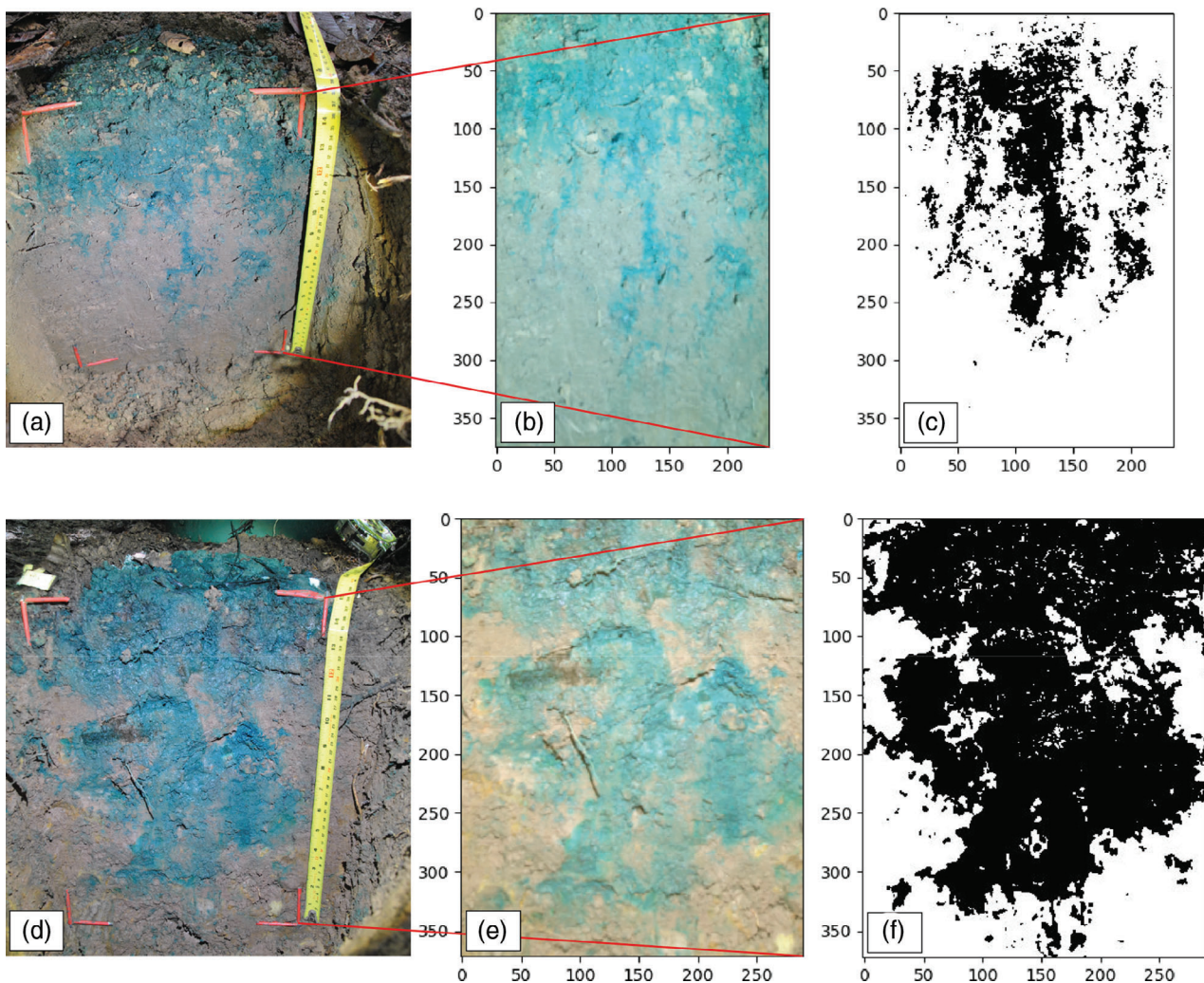
Fig. 1. Example of the steps for processing the photographs of the blue dye experiments. (a) and (d) the original photos with marked corners and tape measure. (b) and (e) the original photographs cropped to the corners of the rectangle and georectified based on location of the markers. (c) and (f) the colour-coded cropped and georectified pictures: black pixels where blue dye is present and white pixels where no dye is present. The images (a–c) were taken below mesocosm 6E (soil-only treatment; day 5); images (d–f) were taken below mesocosm 1D (dung beetle treatment, day 5). Distances in the georectified images are in mm.

Regarding the width of infiltration pathways, there was no visual difference in the distribution of the relative occurrence of the stained path width categories for the treatments or time periods (Fig. 4). The blue dye patterns were dominated by the narrow (0–20 mm) stained path width, with a relatively higher frequency of stained path widths between 20 and 200 mm within the top 100 mm of soil than below, except for one dung beetle mesocosm on day 5 (1D). Large stained path widths (200–1000 mm) were only found occasionally in the uppermost 100 mm of the soil. According to the relative occurrence of stained path width categories (proportion of narrow (0–20 mm) stained path widths: >50%; and proportion of large stained path widths (200–1000 mm): <20%), the flow type was classified as 'macropore flow with low interaction' (Weiler & Flühler, 2004) for 97% of all 10-mm soil depths increments (regardless of treatment or time point).

Discussion

Effect of dung beetles on infiltration rates and pathways

Taking into account the sample size and other varibles it was found that dung beetle activity increases the saturated hydraulic conductivity K_{sat}. This became evident after dung beetles had been active in the soil for more than 5 days. This time frame is longer than that suggested by Brown *et al.* (2010) for a grazed catchment in South Africa, who found that dung beetles increase infiltration rates after 2 days.

Infiltration was dominated by 'macropore flow with low inter-action' in all mesocosms. This indicates that water flows in both macropores, and in soil matrix characterised by low permeability or already saturated condition (Weiler & Flühler, 2004). Macropore flow is expected for clay-rich soils where infiltration occurs
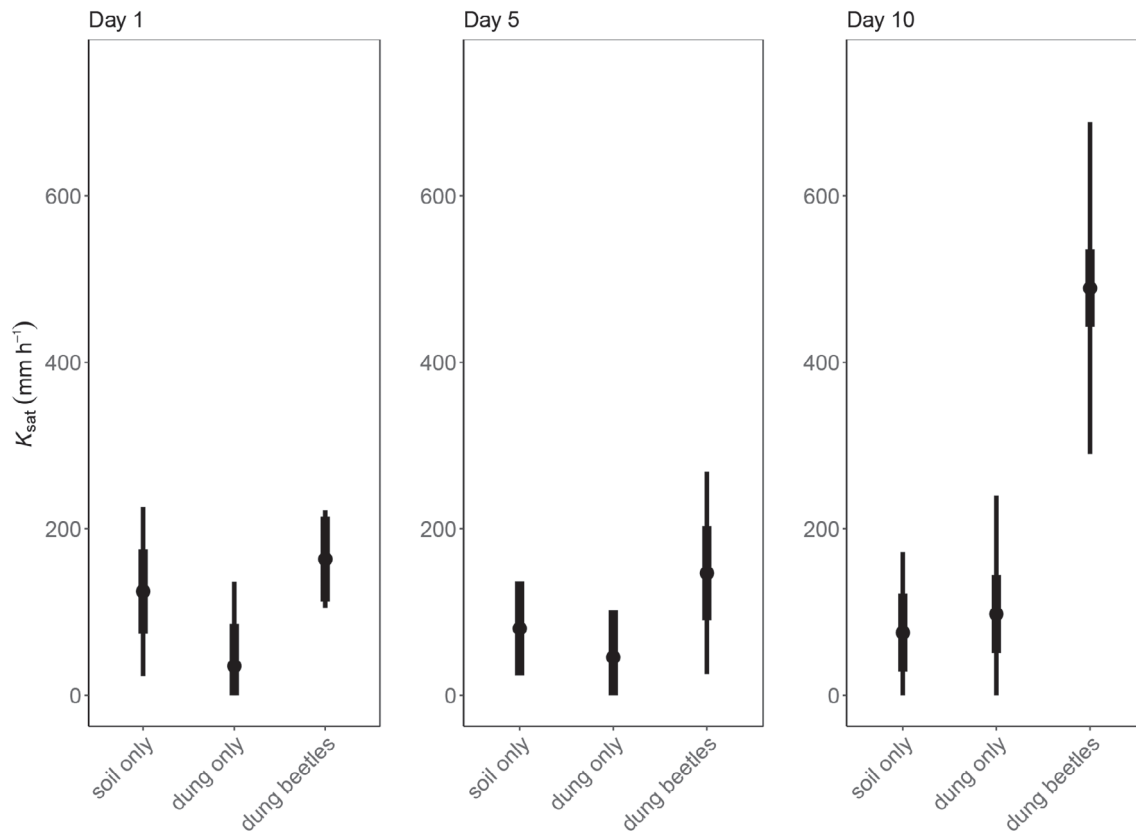
Fig. 2. The average steady-state infiltration rates (K_{sat}) for the different treatments (soil-only, dung-only, and dung beetles) and time periods (day 1, day 5 and day 10; points), with the least significant differences (LSDs) centred around the mean (thick lines), and the 95% confidence intervals (thin lines). Treatments for which the LSDs do not overlap are considered to be significantly different. Note that negative K_{sat} values for the lower confidence intervals are physically not possible and were therefore set to 0.

predominantly via preferential flow paths caused by roots and macrofauna activity (such as tunnelling). Flow in soil matrix under low permeability and saturated conditions is also expected because of ponded conditions during the experiments. Dung beetles did not change this dominant flow type, nor the width of infiltration pathways. There was also no difference among treatments, nor time periods, in the fraction of pores that were stained in the uppermost 150 mm of the soil, where the majority of infiltrated water remained. However, dung beetles appeared to increase the depths to which water infiltrated in the soils; after 5 days, there were more blue dye-stained pores between 150 and 400 mm for the dung beetle treatment compared to the soil-only (day 1 and day 5) and initial dung beetles (day 1) treatments. Although there are no published studies documenting burial depths for Southeast Asian dung beetles, tunnelling depths of up to 50 cm have been observed for similar-sized beetles in Brazil (Gregory *et al.*, 2015), and we observed *Catharsius* spp, in tunnels at approximately 30 cm depth (E. Slade, pers. comm.). The blue dye findings suggest that deeper infiltration may be responsible for the higher K_{sat} values for the dung beetle treatments. Furthermore, these findings also support the results of the infiltration measurements in that the influence of dung beetles is not immediate. While there was little effect of dung beetles on the infiltration patterns after 1 day, by day 5, more

of the soil was stained by blue dye, indicating the wetting of a larger soil area. This suggests that dung beetles expanded their tunnelling network between day 1 and day 5. This may also indicate that dung beetles were building nesting tunnels rather than just feeding on the dung.

These results agree with previous studies highlighting the role of soil macrofauna in creating macropores, thus influencing soil hydrological ecosystem functioning (Hanson *et al.*, 2004; Brown *et al.*, 2010; Colloff *et al.*, 2010; Forgie *et al.*, 2018). While bioturbation caused by earthworms and its effect on soil infiltration has been thoroughly described (Weiler & Naef, 2004; Capowiez *et al.*, 2014; van Schaik *et al.*, 2014; Taylor *et al.*, 2019), less is known about the effect of insect bioturbation. There have (to our knowledge) only been five studies on the effects of dung beetles on soil hydrology (Brown *et al.*, 2010; Johnson *et al.*, 2016; Doube, 2018; Forgie *et al.*, 2018; Cheik *et al.*, 2019). Our study, therefore, contributes to the limited work on the role of insects on infiltration, which is particularly scarce for tropical ecosystems (though see Cheik *et al.*, 2019 for a study in Vietnam). In addition, this study is the first to use blue dye staining, a commonly used technique in soil hydrological studies, to investigate the role of dung beetles in creating macropores and increasing infiltration. Our study demonstrates that blue dye can be a promising tool to evaluate the functional
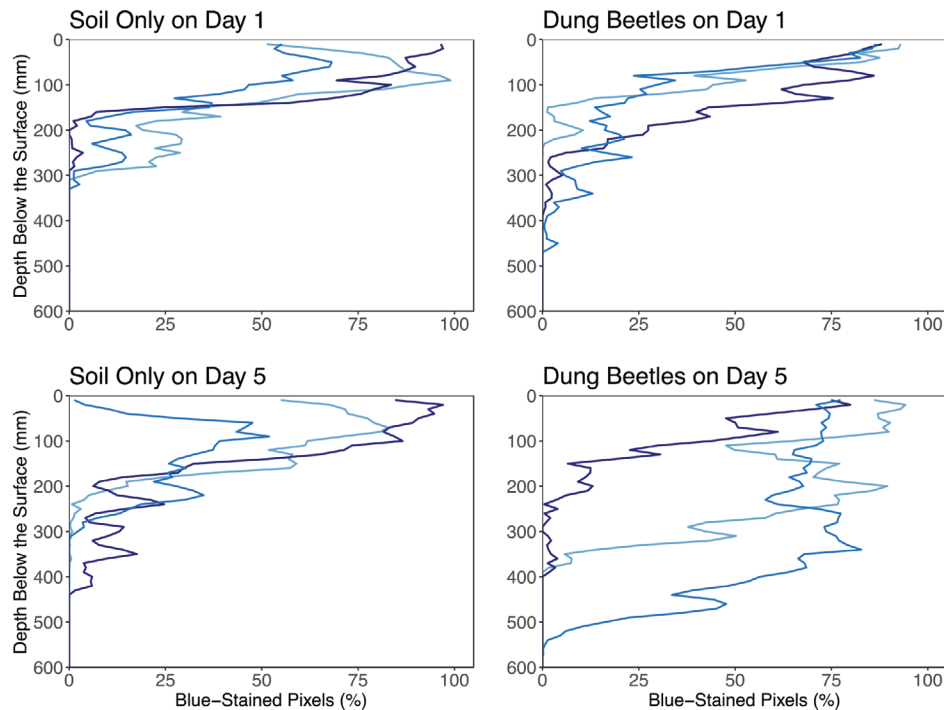
Fig. 3. Percentage of blue dye-stained pixels (%) as a function of depth below the soil surface (mm; averaged per 10 mm depth) for the soil-only (left column) and dung beetle (right column) treatments on day 1 (top) and day 5 (bottom). Each coloured line represents a separate mesocosm ($n = 3$).

effects of macrofauna tunnelling behaviour and opens up the possibility to use this approach to investigate impacts of dung beetles in restoring degraded and compacted soils.

Limitations and further work

Due to our experimental design, we were limited in the number of individuals we could add to the mesocosms and were restricted to only using tunnelling species. Thus, the abundance of beetles used in the experiment may have been lower than those which would have been attracted to naturally occurring dung piles. For example, Slade *et al.* (2011) reported dung beetle abundances at cattle dung baited pitfall traps ($n = 5$) within this area of forest of mean individuals per trap = 31 ± 5 SE, range 22–48). However, our experimental approach, using standardized dung beetle groups, ensured comparable conditions among mesocosms so that our results could be compared directly, and if anything, are conservative. This suggests that a stronger effect of dung beetles may be observed when dung beetles are present at higher densities but further work investigating the impacts of different species and functional groups of beetles under varying densities is needed.

The infiltration measurements were made under ponded conditions. Ponding can cause activation of flow in macropores that do not transmit water during (more natural) nonponded conditions, thereby leading to artificially high infiltration rates. The lack of the double ring and thus the inclusion of lateral flow pathways may have caused the K_{sat} values to be overestimated (Tricker, 1978). However, we assumed that this effect

was relatively similar for all mesocosms and therefore will not affect the comparison of the results among treatments. We also acknowledge that our replication of mesocosms was fairly small and that due to high spatial variability in infiltration rates in forest soils, more infiltration measurements are needed to increase the reliability of the K_{sat} estimates (Zimmermann & Elsenbeer, 2008).

Nonetheless, the observed differences between mesocosm treatments provide a clear indication of the effects of dung beetles on infiltration rates. How long this effect lasts, and what this means for infiltration capacities during regular or extreme rainfall events at the landscape scale requires further studies. The results of the sprinkling experiments by Brown *et al.* (2010) in South Africa suggest that in pasture systems, the effects may last for 6 months. The recorded mean infiltration capacity (48 mm h^{-1} prior to the treatment) was generally lower than the values obtained in other secondary rainforests (between 233 up to 939 mm h^{-1} (Zimmermann *et al.*, 2006; Hassler *et al.*, 2011; Zwartendijk *et al.*, 2017). Yet, we estimate that all rainfall is likely to infiltrate into the soil even during high rainfall intensity events (about 50 mm h^{-1} with the likelihood of occurrence (i.e., return period) of 2 years (Noor *et al.*, 2018) unless the soil is already waterlogged, or during the peaks of the most intense storms. Our findings might therefore be more relevant for degraded sites or sites where soils have been compacted by human activities, such as timber harvesting with heavy equipment, trails, or recreational areas (Hattori *et al.*, 2013). For these compacted soils, the infiltration capacities are likely to be lower, and the risk of overland flow is larger (Mohammadshirazi *et al.*, 2016). Bioturbation, as one of the main mechanisms
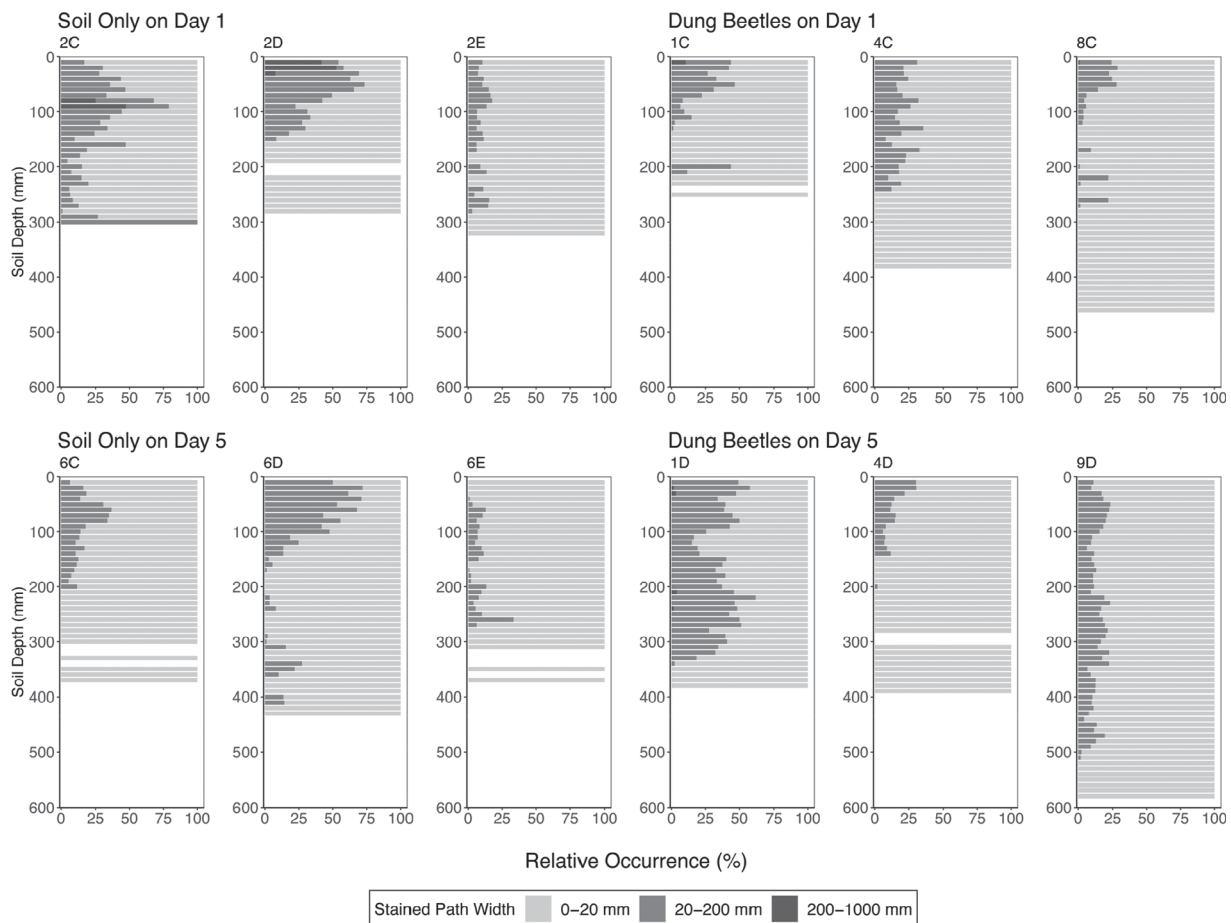
Fig. 4. Relative occurrence of the stained path width categories (*x*-axis) averaged per 10 mm soil depth (*y*-axis). Each plot represents an individual mesocosm with either the soil-only (left) or dung beetles (right) treatment on day 1 (top) or day 5 (bottom). The image processing did not detect any blue dye for some depth intervals, which is indicated by a blank row.

that results in preferential flow pathways in dense soils, could lower that risk and improve infiltration and reduce the amount of overland flow.

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Author Contributions

This project was designed, analysed, and written by NK, ES and IvM. The data were collected by NK and LYC. The manuscript writing was supported by JG, CDP and EG.

Data availability statement

The data that support the findings of this study are openly available in GitHub at https://github.com/NadikeNK/Influence-of-dung-beetles-on-infiltration.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Map showing the approximate locations of meso-cosms in the study area. The symbols represent the experiments: infiltration rate (Ksat), blue dye (BD), infiltration and blue dye (K_{sat} and BD). The colours indicate the treatment (soil-only,

dung-only, dung beetles), whereby the shade of blue (dung beetle treatment) indicates the time step (day 1, day 5 and day 10) for which the measurements were taken. The text in the brackets next to the soil-only mesocosms indicates the time of the blue dye experiments. Note that the GPS coordinates were only recorded for each mesocosm cluster; the location of the individual mesocosms is estimated. Even though the blue dye measurements for the soil-only treatment on day 5 were taken on a hill close to the river (but approximately 2 m above the river), we did not observe any visual differences in the soil type or texture compared to the other locations

Figure S2. Boxplots of the saturated hydraulic conductivity (K_{sat}) for the different treatments (soil-only, dung-only, dung beetles) prior to the treatments (day 0) and the different days after the treatments started (day 1, day 5 and day 10). K_{sat} was repeatedly measured in the same mesocosm for the soil-only and dung-only treatments; K_{sat} measurements for the dung beetle treatments, however, were taken in a different set of mesocosms for each time step (see methods). The dots represent the individual measurements, the box represents the 25th–75th percentile, the line the median, the whiskers extend to the first (0–25th percentile) and fourth (75th–100th quantile) quartile, and the small dots are outliers. Significant differences between K_{sat} measured prior (day 0) to the experiment and after the application of treatments on day 1, day 5 or day 10 are indicated by the horizontal line and the obtained *p*-value above.

Figure S3. Percentage of blue dye-stained pixels: means (points) and least significant differences (LSDs; vertical bars) for the soil-only and dung beetle treatments on day 1 and day 5 for different depths below the soil surface. Note that percentual values above 100 or below 0 are physically not possible, and the confidence intervals (thick lines) were therefore cut above 100 and below 0.

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