

Supplemental Information

Title: Excavation and aggregation as organizing factors in de novo construction by mound-building termites

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Table of Contents

- 1. Experimental setup**
- 2. Tracking**
- 3. Statistical analyses**
- 4. Simulations**

1. Experimental setup

We conducted experiments in Petri dishes 87mm in diameter, using dishes with high walls (25mm) to prevent termites from climbing out of the arenas. Each dish was placed inside a clear acrylic box that prevented airflow around the arena and supported an overhead camera. Experiments were filmed from above using an iPod Touch, which shoots 1080p HD video at 30 fps. We lit the arena using two LED lamps, diffusing the light using a light tent to provide even illumination and prevent shadows and reflections (Figure 1C in the Main Text). Video S2 shows a short clip from one of our experiments.

2. Tracking

We tracked termites throughout portions of each experiment using custom tracking software built in Matlab R2014b (Figure S1). Because our interest in this study is in the process of how termites begin excavating and building in an originally featureless environment, we tracked each experiment for a time window around the first excavation that occurred. We tracked experiments for three minutes before the initial excavation event (or, in cases where the first excavation began less than three minutes after termites were introduced, for the full period between introduction and initial excavation) and up to ten minutes afterward. Because the termites began to disappear under the soil over time, making even manual tracking and consistent identification of individuals impossible, tracking times ranged from 5.3 minutes to 10.0 minutes, with a mean tracking time of 8.3 minutes (we scaled behaviors to account for these discrepancies; see below). In order to increase the speed of tracking, we down-sampled videos from 30 fps to 10 fps; due to the relatively slow movements of termites, this did not reduce the accuracy of tracking.

The user seeds the tracking program at the desired starting frame of the video by manually marking the body and head positions of each termite to track (we excluded those that remain motionless for the entire experiment, as determined by manual inspection of videos before tracking). From this point, the tracker uses background subtraction, a simple motion model that assumes termites will move along the same trajectory as in the previous two frames, and a convolution mask corresponding to the shape of a termite, to identify the body and head positions of all tracked termites at every 0.1s, based on that termite's position in the previous frame.

The tracker relies on manual intervention to ensure accuracy, and has two types of user prompts: correction and approval. The correction prompt appears whenever the tracker self-identifies any of several hard-coded error conditions, such as when two termites are too close together to be automatically separated or when a termite's head and body positions as identified by the tracker are too far apart. The user can edit the tracker's output if there is actually an issue with a termite's position or identity, correcting previous frames as well if necessary. The approval prompt occurs after every minute of tracked video, and presents the user with an image of the current frame overlaid with the positions of each termite according to the tracker. This screen is to ensure that even if an issue does not get caught by any of the hard-coded error conditions, it will be noticed before creating too many tracking errors. The user can search this screen for any issues with the positions and identities of all termites. It is possible through this interface to correct issues on the present and all previous frames.

We used the resulting trajectories, consisting of the positions and orientations of each termite at 0.1-second intervals throughout the tracked time period, along with manually marked excavation sites in the arenas (see below), to identify termite behaviors. Based on the activity we observed, we classified termite activities into the following behaviors (Video S3):

- Wandering/resting: moving around or resting in the arena, away from excavation sites and without carrying soil. This is the most common behavior observed.
- Excavating/building: digging and moving soil around within an excavation site.

We identified the excavating/building state by considering every period in which a termite encountered and remained at an excavation site, as follows. We manually marked the polygonal boundaries of all excavation sites in the dish (Figure S1B). The tracker uses these boundaries, in conjunction with the tracks of individual termites, to identify all instances where a termite's head was within an excavation site region for at least one full second. Each of these periods is then presented to the user to verify if the termite is actually excavating (Figure S1C). In order to make our definition of excavating less dependent on the manually drawn borders of excavation sites, we merged together excavations in which a termite left and returned to the same site within thirty seconds while staying within two centimeters of the site.

When a termite is not working at an excavation site, its behavior is classified as wandering/resting. Termites in this state typically either walk around or stand still somewhere in the arena. Since we are not evaluating the behaviors of termites not engaged at an excavation site, we do not distinguish between termites that are resting and those that are wandering.

Figure S2 displays the full trajectory for a single termite during the course of an experiment, with its track classified based on its behavior at the time. We note that termites spend the majority of their time around the edges of the arena and most excavation sites likewise appear along the edges.

Identifying and estimating depositions

Behaviors within excavation sites were often obscured in the videos, making it impossible to identify every time termites deposited soil while working inside an excavation site. We estimated deposition activity by manually observing the end of each of the 271 excavation events in our experiments. We observed depositions occurring at the end of 122 of the 271 events, identifying them by watching for the head-screwing motion that termites make when placing soil as well as the appearance of a new pellet against the surface of the arena. Transport distance was calculated as the linear distance between the center of the source excavation site and the deposition location. The majority of the observed depositions occurred within excavation sites (87/122), while the rest were carried away from the excavation site and deposited later (35/122). By the nature of our observation process, we observed every time a termite transported soil away from an excavation site, but only a subset of depositions that occurred within sites (because termites often went through multiple excavation-deposition cycles before leaving a site). We therefore took the depositions that occurred within excavation sites at the end of an excavation event as a representative sample of those that occurred during excavation events but could not be observed. We estimated the total number of excavations based on the cumulative duration of excavation activity (572.6 minutes) together with the typical time observed for the individual operations of picking up and depositing a soil pellet. To evaluate the latter, we measured two excavation-deposition cycles from each trial, one in the second minute of excavation and one in the eighth, for a total of 36 samples each for excavating and for depositing a pellet. This gave an average of 24 ± 10 seconds to excavate a pellet and 9 ± 5 seconds to deposit the pellet, meaning it took 33 seconds for a full cycle. This implied that there were a total of

$\frac{572.6 \text{ min}}{0.55 \text{ min/cycle}} = 1,041$ deposition cycles during the course of our experiments. We assumed that

the distribution of transport distances for the 87 manually marked within-site depositions was

typical of the distribution for all of the within-site depositions estimated to have occurred, and bootstrapped our observed sample to obtain a sample for the 1,006 within-site transport distances (mean distance = 1.4 cm). We then merged that sample with the distribution for the 35 outside-site depositions (mean distance = 4.9 cm), to yield an overall mean of 1.5 cm for average linear distance a pellet was moved between excavation and deposition. Note that the vast majority of depositions occurred within or in the immediate vicinity of excavation sites: in only 35 cases (3.4% of all depositions) did termites carry a pellet beyond the vicinity of an excavation site.

3. Statistical analyses

In this section we describe how we fit statistical models to the tracking data in order to infer what stimuli influence key termite behaviors of interest: whether they initiate a new excavation site while wandering, whether they switch from wandering to excavation on encountering an excavation site, and how long they continue excavating before leaving a site. First, we had to specify the variables and modeling approach. We investigated the effects of multiple potential stimuli on termite behavior that fall into three categories; these are the fixed effects of our study:

1. **Traits of individual termites:** These features suppose that individuals have unique traits that affect their behaviors.
 - a. *Excavation propensity:* The total amount of time the termite spent excavating during the experiment. This feature supposes that some termites may be characteristically more prone to dig than others. Since termites vanishing under the soil made ongoing tracking impossible less than ten minutes after the first initiation in some trials, not all experiments were tracked for the same amount of time. We accounted for this discrepancy by scaling each termite's excavation activity to match a 10-minute tracking period. For example, if a video was tracked for 9 minutes and a termite excavated for 4.5 minutes, we scaled this value to show that the termite excavated for 5 minutes out of 10.
 - b. *Activity level:* The total distance the termite traveled during the experiment before the first excavation site was initiated. Similar to excavation propensity, this feature supposes that some termites may be characteristically more active than others. In the same way as in (a), since not all experiments were tracked for exactly three minutes before any excavations, we scaled each termite's activity level as if each experiment was tracked for three minutes before any initiations.
 - c. *Species:* A binary value according to whether the termite's species is *M. michaelseni* (0) or *M. natalensis* (1). This feature allows us to evaluate any behavioral differences between the two species in our study.
2. **Past behavior of individual termites:** These features are based on the hypothesis that a termite's past behavior (e.g., memory of previously excavating at this or other sites) may influence the decisions it makes in the present.
 - a. *Site initiator:* A binary value according to whether this termite was the one to have initiated the current excavation site. This feature considers the possibility that the initiators of a site act differently than non-initiators.
 - b. *Previous excavation at site:* A binary value according to whether this termite had previously excavated at the current excavation site. This feature takes into account the possibility that a termite might behave differently upon returning to an excavation site than upon first encountering it.
 - c. *Previous excavation at any site:* A binary value according to whether this termite had previously excavated at any excavation site (including the current one). This feature considers the possibility that a termite might behave differently before and after having engaged in excavation during the experiment.

- 3. Features of excavation sites:** These features hypothesize that termites are affected by the current characteristics of excavation sites they encounter.
- a. *Number of termites:* The number of other termites actively working at an excavation site when a termite encounters the site. This feature considers how a termite is affected by the presence of other working termites.
 - b. *Cumulative site activity:* The total amount of excavation that has occurred at a given excavation site (measured as the total time spent excavating there by all termites up until that moment). This feature could be a proxy for the size of a site or the amount of a chemical that has been left there.

Based on the design of our experiments we also had multiple random effects. Our experiments contained blocked samples: each of the six mounds supplied termites for three trials, each of which contained 25 termites, and Analyses 2 and 3 contained multiple measurements of some termites. It is necessary to account for these repeated measurements so as to avoid pseudoreplication and potentially misleading results (1). These variables—colony, trial, and individual—therefore constitute random effects. Note that because we are interested in the effect of species on behavior and there are only two species in this study, we treat the species of each termite as a fixed effect.

Because our models contained both fixed and random effects, we identified parameters using linear mixed models (LMMs) and generalized linear mixed models (GLMMs), using the *lmer* and *glmer* functions in the R package *lme4* (2). GLMMs were fit using the Laplace approximation (1, 3). We identified the best model by performing backwards selection using the Bayesian Information Criterion (BIC) (4). The BIC rewards models for accuracy while penalizing them for complexity; it therefore provides a metric to determine the most effective yet parsimonious model. We began with a full model that included all applicable fixed effects along with interactions of these fixed effects with species, and performed backward selection by considering the impact of removing each individual fixed effect from the model, iteratively removing the feature that most improved the BIC until it was impossible to improve the BIC by removing more features.

4. Simulations

To compare how construction processes might progress if termites were driven by only a single primary mechanism, we created agent-based simulations based on behaviors we observed in our experiments, and compared their results to those of simulations based on prior models that emphasize pheromone-based depositions. In the “excavation model,” agents are focused on digging: the primary driver is for agents to join others in excavating at a common site, with deposition a secondary effect as agents drop material anywhere nearby. In the “pheromone model,” agents are focused on deposition: the primary driver is classic stigmergic building, with agents influenced by a cement pheromone to deposit material in the vicinity of other recent deposits, and excavation a secondary consideration as agents pick up material at random. Note that these simulation models do not attempt to capture the observed insect behavior in full detail; instead, their purpose is to provide a broad idea of what patterns of excavation and deposition appear if agents are driven by a single mechanism of interest.

The circular arena was modeled as a grid of lattice cells each corresponding to a pellet of soil. Pellets were taken to be cubes 1 mm on a side; the arena was 87 mm in diameter, with an initial depth of 4 mm. Termites were represented as rectangles 8 mm x 3 mm, with continuous-valued position and angle. If not already carrying a pellet, and if the point at the location of its mouth (the center of the rectangle’s leading edge) had not already been excavated down to the bottom of

the dish, an agent could remove the top pellet from that point and begin carrying it; if carrying a pellet, an agent could deposit it atop the stack at the point at the location of its mouth. We set the time it takes an agent to excavate and to deposit a soil pellet by timing a sample of termites performing these actions from our experiments (see *Identifying and estimating depositions*, above); these observations provided deterministic durations for excavating (24 sec) and depositing (9 sec) soil in the simulations. In each trial, 25 agents were initially scattered at random, and the trial proceeded for 10 simulated minutes after the moment of first excavation. Time steps were 0.1 second. We performed 100 trials for each model to collect statistics.

Motion model

Both models incorporate a “wandering” state that describes the motion of termites exploring the dish or otherwise not engaged in building. A wandering termite uses a motion model with four substates: resting (no motion), turning left (at a rate of $3^\circ/\text{step}$), turning right ($3^\circ/\text{step}$), and moving forward (2mm/step, with angular displacement per step chosen from a normal distribution with mean 0 and standard deviation 4.5°). These displacement values, and the probabilities of transitions between substates, were chosen based on quantitative observations of the movement of termites in our experiments (before excavations began). We used the tracking data to measure the average speed, turning rate, and linear and angular distances moved before stopping or switching to a different motion. We set the parameter values of the model to approximate the average values of these behaviors, as measured in the tracking data. The table below gives the transition probabilities between motion substates.

↓From →To	Rest	Left	Right	Forward
Rest	0.6	0.1	0.1	0.2
Left	0.3	0.5	0	0.2
Right	0.3	0	0.5	0.2
Forward	0.4	0	0	0.6

Table S1. Markov transition matrix between the substates of the wandering motion model

Collisions are detected if the rectangle representing an agent intersects with the arena boundary or another agent. An agent whose movement during a time step would cause a collision makes no movement instead; if in the wandering state, it switches to the resting substate. When colliding with the arena boundary, an agent rotates 10° in the direction that takes it closer to parallel with the boundary, as long as doing so does not result in a new collision (again based on observations from our experiments, in which termites that reach the dish wall tend to turn to align themselves with it). Based on sideways movements observed in our experiments, we also let collisions with the boundary or other agents push an agent 1 mm laterally, if this did not result in another collision.

In our experiments, we observed that when blocked from advancing by the presence of others, termites frequently retreat and head in the other direction. Accordingly, we implemented a corresponding routine for agents in the model: if an agent is unable to move for 5 seconds via any of the above actions, it backs straight up (at 2mm/step) for one second and then rotates 180° over 1.5 seconds before returning to normal behavior. We also observed that termites engaged in excavating at the same site would frequently squeeze past or climb over each other to reach the excavation. Accordingly, we relaxed the collision detection in the excavation model for agents in the excavating state: collisions with arena boundaries were treated as above, but collisions with other agents were ignored. An agent in the wandering state would still be subject to colliding with agents in the excavating state; as a result, excavating agents provided an obstacle limiting others

from joining a site (Figure S3). When an excavating termite returned to the wandering state, collisions with other termites would start being registered again beginning with the first time step at which that agent's rectangle had no intersections with others.

Excavation model

Termites in this model can be in the wandering state described above or in an excavation state (Figure 3A). The latter involves a four-stage cycle: (1) choose a place from which to remove material; (2) excavate a pellet (24 seconds); (3) find a place to deposit the material; (4) deposit the pellet (9 seconds).

A wandering agent can transition into the excavation state in two ways (Figure 3A):

1. If its mouth is at a point where no excavation has yet occurred, it can initiate an excavation site there with probability $P_{initiate}=2.4483e-05$ per time step (i.e., one frame or 0.1 seconds). This value was determined based on the frequency with which termites in our experiments initiated a new site during a particular time step, i.e.,

$$P_{initiate} = \frac{N_{sites}}{N_{termites} \cdot N_{frames}} .$$

2. If its mouth comes within 3 mm of an excavation, it has a probability $P_{join}(n)$ of joining the excavation at that site, where n is the number of other agents currently excavating at that site. (Lattice cells within 2mm of each other at which excavation has occurred are considered to be part of the same excavation site.) The value of this function was determined based on the values from the GLMM analysis corresponding to Analysis 2 in the main text, but where the only dependent variable was the number of agents excavating, with all other features held at their mean observed values (Figure S4). If the agent does not join a site when first encountering it, it will not reconsider joining that site until after it has left the site's vicinity and returned.

An agent initiating a site begins digging there immediately. An agent joining an existing site chooses a point at which to dig by looking within a distance of 4 mm from its mouth and selecting the deepest point at which no other agent is currently digging. If the deepest such point is already excavated to the bottom of the dish, it chooses an adjacent cell. In the case of ties, it chooses the closest such point to its current mouth location. It then proceeds to that point before starting to dig. If another agent starts digging at that point before it reaches it, it selects a new point in the same way.

Once an agent has a pellet, it looks for a nearby place to put it. To do so, it chooses one of {move forward, move backward, turn left, turn right} and proceeds in that direction until its mouth reaches a cell 2 mm from any excavation. If it takes it more than 2 seconds to find such a cell (e.g., because the arena boundary limits its movement in that direction), it chooses another direction at random with equal probability. Upon finding such a cell, it begins depositing its pellet there.

Note that this process excludes the possibility of soil pellets being transported long distances away from the source excavation site. Because we do not believe that long distance depositions are essential to the building process at this early stage, based on their rarity in our experimental observations, we did not include them in the simulations. The simulation results, in which we find that the excavation model resembles our experiments more closely than does the pheromone model, suggest that long distance depositions are not essential in the observed de novo construction.

We modeled transitions between the wandering and excavation states as a two-state Markov chain, in which there was a fixed probability that a termite in the excavation state, after completing each excavation-deposition cycle, would remain in that state and excavate and deposit another soil pellet. We used nonlinear least squares estimation to determine this probability: as described above, we measured excavation-deposition cycles as taking on average 33 seconds. Based on the distribution of excavation durations (Figure S5B), we determined the frequency with which termites completed a given number of continuous cycles, using nonlinear least squares to find the continuation probability that best fit this data. We found that an agent has a probability of $P_{continue}=0.70$ to continue excavating for another excavation-deposition cycle at the same site, while with probability $1 - P_{continue}=0.30$ the agent transitions to the wandering state.

Pheromone model

Agents in this model (Figure 3B) excavate at random and deposit as influenced by a transient pheromone that they leave along with deposited material. We implemented the structure and parameter values of this model following a data-driven simulation model for *Lasius niger* (5), due to the similarity of the structures during early building for both insects and the availability of model parameter values based on experimental observation.

The probability that a wandering agent begins excavating is given by $0.0029/k$ per time step, where $k = \max\{1, \text{the number of pellets added above the original soil depth}\}$.

Once an agent has a pellet, it moves as it does in the wandering state until it finds a place to deposit it.

The probability of deposition is given by $1 - \exp(-c \cdot \exp(-t/T))$, where c is $0.011k$ for points where previous deposition has occurred and 0.0025 otherwise; t is the elapsed time since the most recent deposition within the Moore neighborhood of the current point; and T is the time constant of the pheromone, taken to be 1000 seconds (5). To prevent deposition from being forbidden everywhere before deposition begins, the arena is initialized with a uniform nonzero level of pheromone present, as though deposition had occurred at every site 10,000 time steps before the trial started.

We also tested an alternative movement model in which laden agents move up a gradient of diffusing cement pheromone before depositing, consistent with certain other pheromone-driven simulation models (6, 7). The results from the simulation designed in this manner were almost identical to the results from our main pheromone model as described above (based on the metrics shown in Figure 2), so we only show results from that version of the simulation.

Figure S6 shows typical outcomes from the experiments and each simulation model. Video S4 displays a timelapse of the building process and a segment of the termite behavior for each of the two simulation models.

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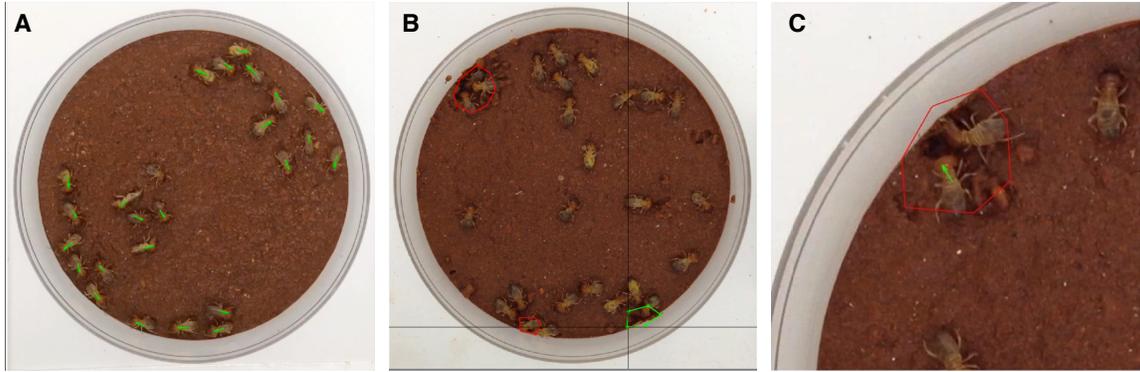


Figure S1. Overview of the tracking process.

(A) The body and head positions are tracked for every termite that does not remain stationary during the entire experiment. (B) Manually marking the boundaries of excavation sites. Red sites are marked and the green site is in the process of being manually marked. (C) A screenshot from a video clip presented to the user to verify whether the termite marked with a green arrow is excavating.

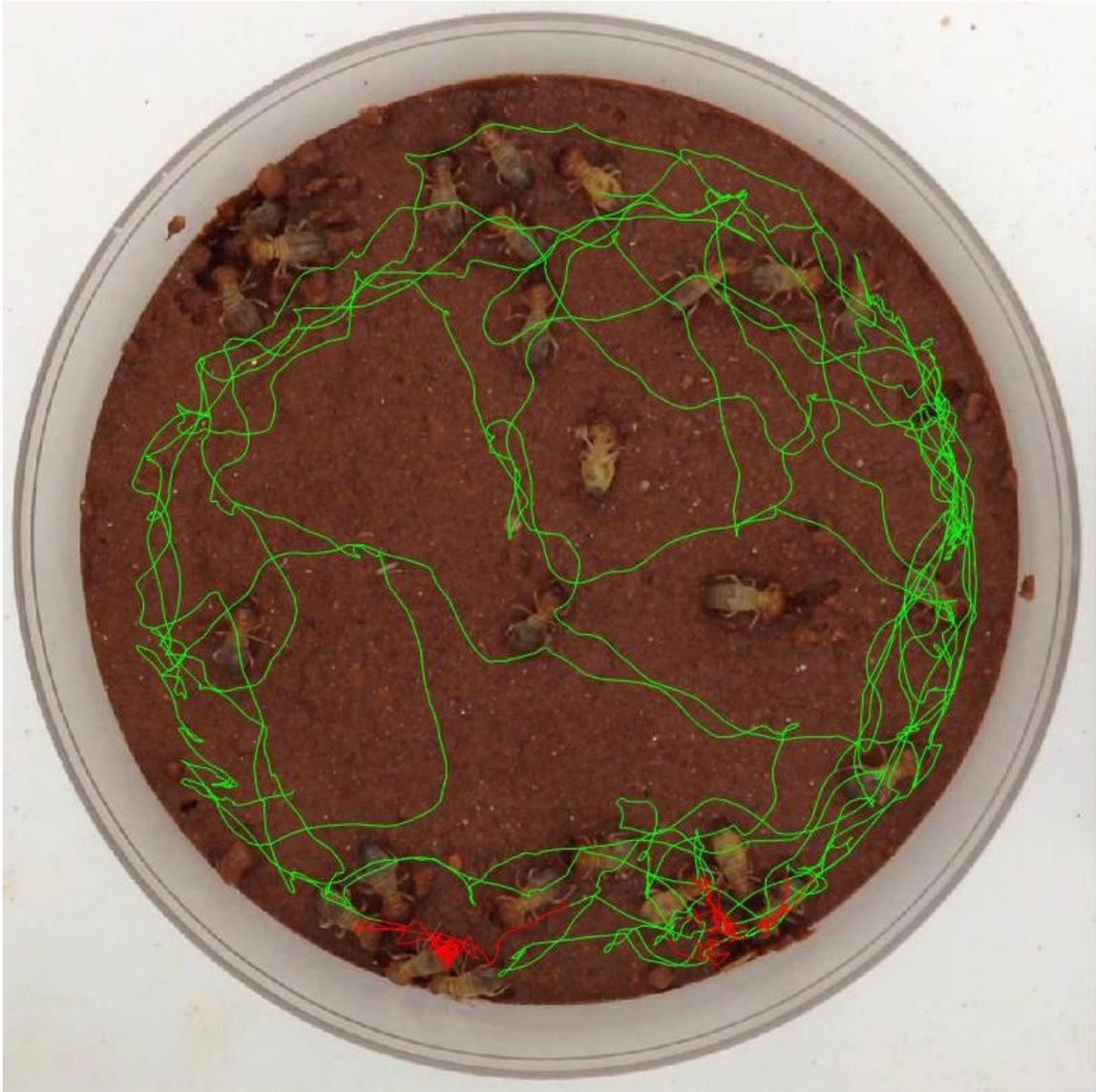


Figure S2. Position and behavior trajectory of a single termite during the course of an experiment.

Wandering/resting is shown in green while excavating/building is shown in red.

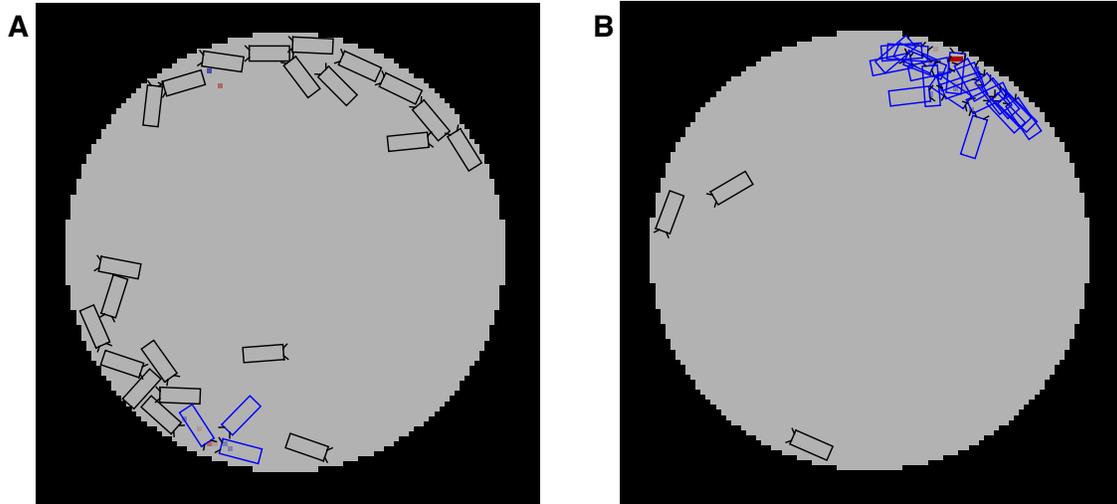


Figure S3. Snapshots of simulated arenas using the excavation model, with and without collision detection.

(A) In our simulation models, wandering termites (black) are prevented from entering the space occupied by others. As a result, the bodies of termites engaged in construction (blue) act as a physical obstacle limiting access to an excavation site, providing negative feedback limiting the number of termites working at any given time. (B) If this restriction is removed and termites are permitted to pass through each other, nearly all end up working simultaneously at the same site.

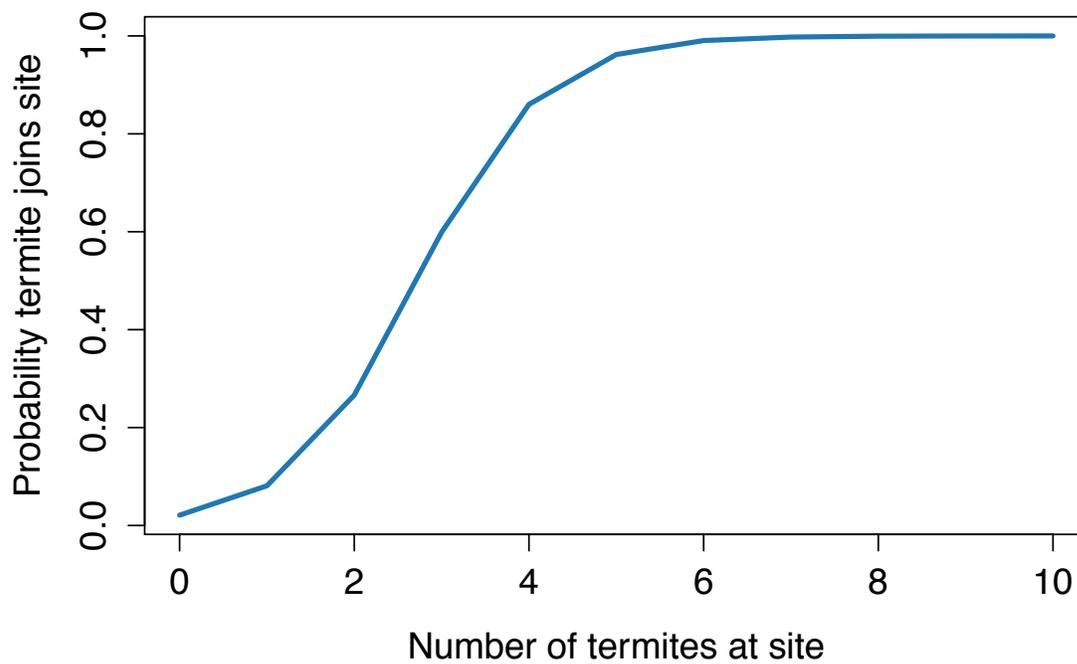


Figure S4. Site joining probability based on the number of termites working there, $P_{join}(n)$. Values obtained from an analysis based on Analysis 2, considering only the number of termites as a fixed effect, with all other factors held constant at their mean values (calculated using the effects package in R).

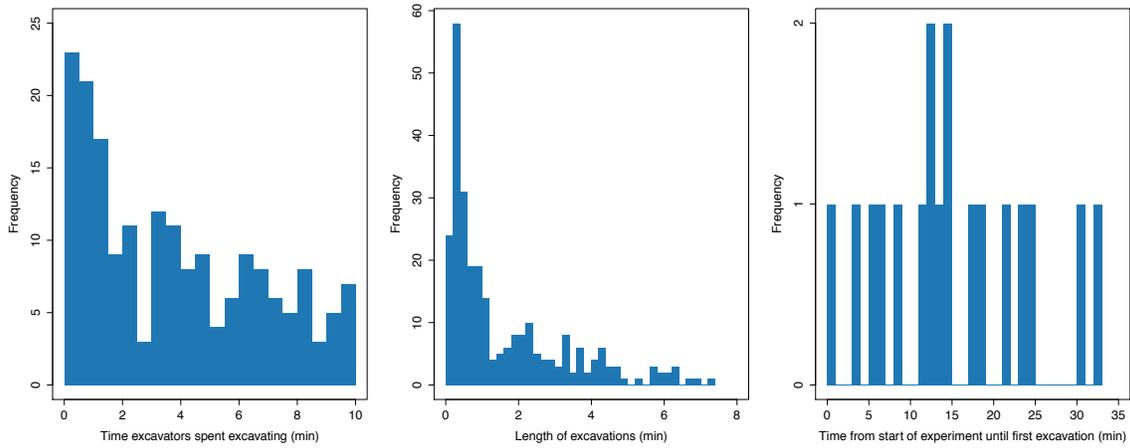


Figure S5. Descriptive behavioral results for excavation.

(A) The distribution of (normalized) time spent excavating among all termites that excavated at any point during our experiments. (B) The distribution of how long each individual excavation lasted. While most excavations lasted under two minutes, the distribution has a heavy tail, with the longest excavation lasting almost eight minutes. (C) The distribution of how long after experiments began the first initiation occurred. The first initiations of each trial occurred at a wide range of times, from the first minute to after the thirtieth minute.

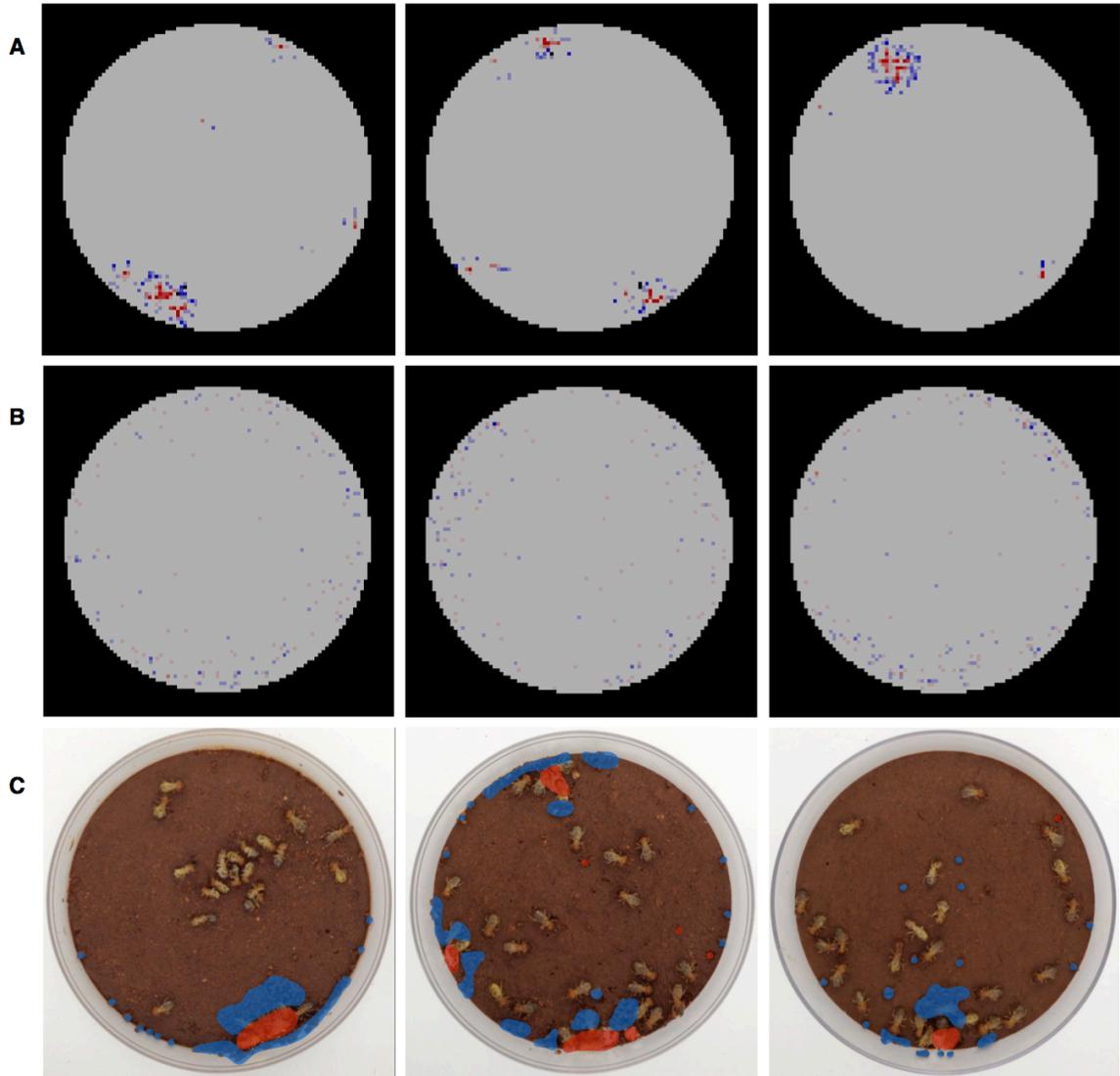


Figure S6. Typical outcomes from the excavation model, pheromone model, and experiments.

(A) Screenshots from the end of three trials of the excavation model. (B) Screenshots from the end of three trials of the pheromone model. Gray represents the base layer of soil. Red and blue represent excavation and deposition locations, respectively, with darker shades identifying a larger extent of digging (i.e., deeper) or building (i.e., taller). (C) Screenshots from the end of three trials, with excavations marked in red and depositions in blue.

The following supplemental videos are available in the Dryad Data Repository:
<http://dx.doi.org/10.5061/dryad.82h58> (8).

Video S1. Sample clip from one of our experiments.

Video S2. Sample clips of the two termite behaviors classified.

Video S3. Timelapse video of termites working over approximately ten hours.

Video S4. Timelapse and building processes from simulations.