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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

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Red imported fire ants (RIFA),*Solenopsis invicta*, have been observed to display significantly different surface construction based on soil moisture in natural soil. To investigate a reason for such a difference, we focused on the pellets used to make surface constructions in 20% and 30% water concentrated soil and observed their sizes and shape by measuring their area, maximum and minimum Feret diameters, and eccentricity. Our results showed that although pellet shape remains relatively constant between moisture conditions in natural soil, the size distribution is affected by the moisture content.

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Chapter 1

Introduction

1.1 Fire Ants,*Solenopsis invicta*

The red imported fire ant (RIFA), *Solenopsis invicta*, are an invasive species that came to United States from South America during the 1930's, through the port of Mobile, Alabama[10]. Since then, RIFA have spread to at least fourteen states, including Georgia, and five other countries, causing significant damage to native ecosystems, agricultural production, human infrastructure, and human health [2, 10].

RIFA are social insects. This means that they live in colonies and display group integration, division of labor, and an overlap of generations. As insects, they have three pairs of legs, one pair per thoracic region. In a colony, the majority of the ants are adult workers. They are red-brown in color, sterile, lack wings, and are generally between $3 - 6$ millimeters in length (see figure 1.1). Workers are assigned tasks depending on their size, with the majority involved in building the nest, finding and distributing food, and defending the colony from outside attacks [10].

RIFA nest building requires a collaborative effort that requires multiple steps. First, workers find a suitable location, determined by biotic and abiotic factors, before excavating tunnels and underground chambers[3, 10]. Simultaneously, using their

Figure 1.1: Picture of RIFA workers in a sand-clay mixture (25% clay, 75% sand, 8% water by weight). Although workers can vary in size, they all share the basic features.

strong mandibles, workers take the soil and form pellets, to create surface structures that eventually become mounds, as shown in figure 1.2 [1, 4, 8, 10]. We define a pellet as an ant made object carried by the ants between tunnel excavation and deposition. The exact mechanism behind pellet formation remains unknown, though previous research suggest that a multitude of factors, such as soil composition, mandible size, and water concentration, influence its creation [4].

1.2 Motivation

Previous observations in Rieser lab suggest significant differences in surface construction behavior as soil moisture concentration changes. As shown in figure 1.3, surface construction appears to vary greatly with soil moisture levels. We are interested in comparing the distribution of ant pellet sizes across selected moisture levels and per-

Figure 1.2: A comparison photo of surface construction found in nature and surface construction found in lab. Note the granularity found in the construction in both photos. Both surface construction involved natural soil and ants found in Atlanta, Georgia.

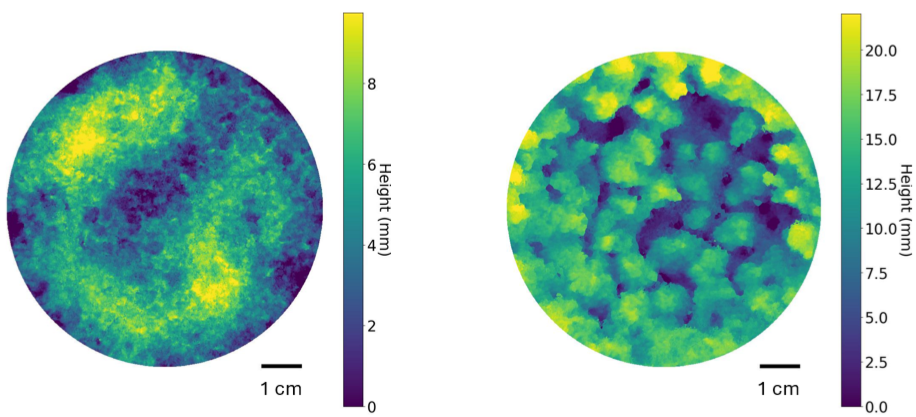

Figure 1.3: 3D heat maps of the height of surface construction in 20% and 30% water concentrated soil, by mass. Both experiments involved ants from the same ant colony (c29), temperature (22°C) and was prepared with the same method (see Chapter 2.2.1). The only difference was the amount of water added to the soil. Construction in 30% water are taller and have more tower-like structures.

haps identifying a relationship between soil moisture and pellet sizes. Previous studies on ant pellets utilized only artificial soil and a small amount of ants, which may not fully represent the dynamics in a natural environment [8]. We aim to explore ant pellets created with natural soil so we can better understand RIFA building behavior and their surface construction in the environment.

Chapter 2

Methods

2.1 Experiment set up

2.1.1 Ant collection and Maintenance

Ant experiments were conducted by Thomas Bochynek, Zhuo Chen, Zach Germain and Emmaline Arter with steps devised by Thomas Bochynek. The experiments were based in an 750ml beaker with 200 g of soil and water (see section 2.2). Ants were gathered from Emory University's Briarcliff campus, with colonies chosen by their proximity to nearby roads, size, and health. Only colonies more than one foot away from roads and trees were chosen to avoid below-road gravel bases and roots. The size and health of a colony were assessed by disrupting the mound and observing the ants' response, with healthy and large colony showing an immediate response and increased movement [10].

To collect the ants, first, the inside walls of collection containers, usually large plastic boxes, were primed with copious amounts of baby powder. This was done by rotating each box and applying a thin coating of baby powder to each face. Personal protective equipment (PPE), consisting of rubber gloves, boots, and long sleeve shirts and pants were worn during the collection process to avoid ants stings. Additionally, baby powder was applied to shoes to prevent ants from climbing. Finally, shovels were used to dig up the ant colony and transfer them into the collection boxes, with care taken to avoid suffocation by adding soil in small batches.

Once collected in the boxes, ants were maintained in the soil. Ants were given approximately 200ml of water everyday, and given a (50 : 50) honey water mixture on a need basis. Ants were only removed for experimental use; otherwise, the ants remained in the containers. Furthermore, the lid to the ant container was left slightly ajar to prevent excess moisture from building up in the containers and removing the baby powder off the walls.

To remove ants from the container for experiments, three additional buckets or containers were used. The first bucket was used to collect the ants from the containers, prepared with baby power in a way similar to the containers. The ants, along with soil, were transferred to the first bucket via a small shovel. The second bucket, filled with water, was connected to the first bucket via a siphoning system composed of a simple plastic tube. The siphon rate was controlled by pressure from a clamp attached to the plastic tube. The siphoning started off slow so the ants in the bucket had enough time to move to higher ground. After roughly ten minutes, the siphoning rate was increased until the bucket was been flooded. Since RIFA are known to float in water, it is possible to simply scoop up the ants [10]. The third bucket was prepared similarly to the first bucket, but PTFE was used instead of baby power. From past experiences, this was the most effective way to prevent the ants from climbing out; the wet ants seem to be able to climb on the baby powered surfaces. After leaving the ants to dry at room temperature, they are ready to be used in an experiment.

2.1.2 Soil Collection

The soil used in the experiments was collected in the same location as the ants, but away from any nearby colonies. This to to make sure that the soil was similar to what the collected ants created their natural nests with, but not part of an active nest. Soil was collected in a similar fashion to the ants; a container, without baby powder, was filled with soil with a shovel. To reduce the amount of grass roots collected, we dug and collected soil 7-13 cm below the topsoil; we did not seek a maximum depth, but we avoided the top few centimeters. In Atlanta, the soil used was red-orange; darker brown soil was avoided. The collected soil was dumped out on a table and dried underneath a heat lamp for 72 hours or longer, depending on the soil moisture level. The soil is then collected and grounded into a fine powder by a model 750 high speed multi-functional crusher. Roughly 1 cup of soil was placed into the blender at a time and pulse grounded for 30 seconds. The resulting powder was then sieved at 425 microns. Only soil below 425 microns are used to control for size. Soil clumps greater than 425 microns were reprocessed until they become less than 425 microns. Rocks and other non-soil elements were discarded and not used.

2.1.3 Camera and Imaging

Images were taken with a FLIR Grasshopper3 GS3-U3-51S5C camera attached to a Kowa LM12FC24M C-Mount 12mm Fixed Lens. The camera was supported by an aluminum beam system, borrowed from Sky Yu's experiments. The aluminum base consisted of 2 5X5cm faced aluminum C-beams at 10.2 and 14.4cm, in length, joined by an an aluminum 2 0.635cm hole L-shaped connector. In addition, two thinner $2x$2cm square faced aluminum c-beams were used as the spine and neck of the aluminum skeleton. The camera was joined to the neck of horizontal neck and faced vertically downward. A neewer nl-288arc LED light board was placed upon the one of the C-beams and was balanced by another aluminum 5 X 5 X 10.2 inch C-beam(see figure 2.1).The lightboard, at maximum brightness, provided a white background that contrasted the red-brown pellets. A 13 cm X 13 cm X 0.3 inch transparent acrylic square was placed on top of the lightboard directly underneath

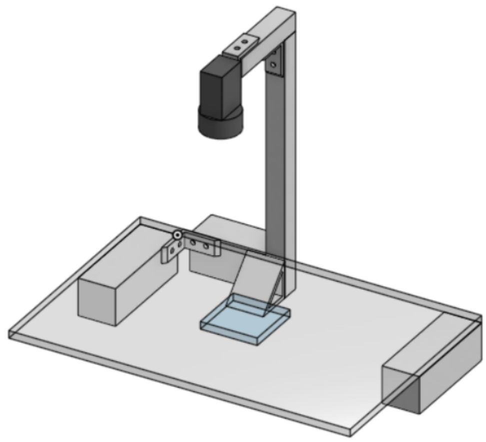

Figure 2.1: 3D view of the experimental set up. The experiment was conducted upon a white light board, supported by 4 boxes. On top of the board lies a vertically aligned camera, created with an aluminum base.

the camera. The acrylic held the sample without dirtying the lightboard. The entire set up was assessed for flatness via a bubble level.

To take the picture, the SpinView software was utilized. Unlike the default camera program, the SpinView software offers enhanced control options such as exposure, gamma, and white balance. To capture high quality images, it was imperative that lighting and color balance to be controlled since we want to capture the distinct traits of individual ant pellets.

Information from the image was extracted in MATLAB [6]. First, images are binarized to black and white via an threshold value. In MATLAB, an image was represented as an array with various values. These values correspond to specific pixel colors. To binarize the array, first the image was changed into a grey scale image. Since 0 is black and 1 is white in MATLAB, in a greyscale image, all values are between 0 and 1; darker pixels have values closer to 0 and lighter pixels have values closer to 1. A threshold value was then chosen in such that any value less than that threshold value becomes 0 and any value greater than the threshold value will become

1. This effectively returns an array that was only composed of zeros and ones.

After the image has been binarized the bwconncomp and regionprops MATLAB functions are used to identify target regions and extract more information (see figure 2.2. For this study, we extracted information regarding the maximum-minimum Feret diameters and area for each ant pellet through MATLAB. In a binary image, represented as an array of 0's and 1's, the bwconncomp function identified white regions by looking for connected 1's in the array. For our purposes, we used the inverted binary image so our regions of interest, the pellets, were white and the background was black. This way, bwconncomp could identify the pellets. Two pixels in MATLAB are considered connected if they share any common edge or corner. Regionprops then measured the area and for each identified region by counting the number of pixels in connected regions. Regionprops also found the maximum and minimum Feret diameters, which represent the longest and shortest distances between two parallel tangents on the pellet's convex hull [9, 11]. Based on investigations of a few pellets, we chose the Feret diameters as the major and minor axes for each pellet because they seemed to better capture the dimensions of a sample of pellets than the major and minor axes associated with an ellipse.

After the area and maximum and minimum Feret diameters for each region were found, it was possible to quantitatively describe the shape and characterises of each region [7]. Eccentricity (e), a measurement of roundness, is defined as

$$e = \sqrt{1 - (\frac{b^2}{a^2})} \tag{2.1}$$

where a and b are the major and minor axis of an ellipse [7]. The closer the eccentricity is to 0, the more circular the ellipse.

By default, MATLAB reports all measurements in units of pixels. To convert the pixels into millimeters, ten pictures of a penny were imaged and the diameter of the penny was calculated ten times. The penny's diameter was used as reference since

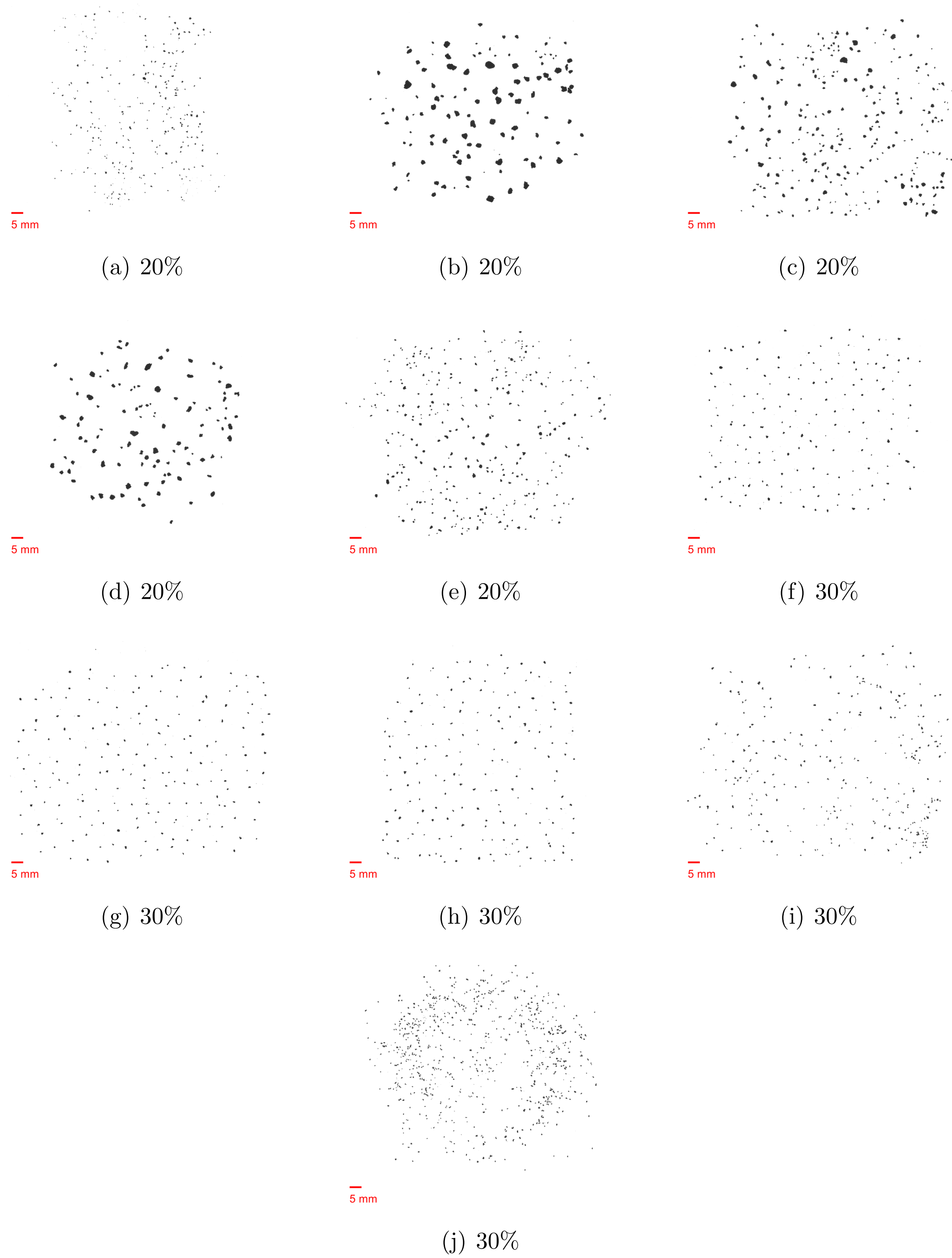

Figure 2.2: Raw images of ant pellets taken with experimental set up. Images a-e are pellets with 20% moisture and images f-j are pellets with 30% moisture. These images were taken with a FLIR Grasshopper3 GS3-U3-51S5C camera attached to a Kowa LM12FC24M C-Mount 12mm fixed focal length lens. The white background comes from the neewer nl-288arc light board. At maximum brightness, the acrylic becomes transparent and only the pellets were imaged.

they can be manually measured. By dividing the true diameter, 19.05mm by the average pixel length measurement for the penny's diameter, 407 pixels, we found that one pixel length roughly equals 0.0468 ± 0.001mm, where the uncertainty is estimated by considering a one-pixel change in the diameter of the penny.

2.2 Preparation and execution

2.2.1 Ant Experiment

In order to image ant pellets, experiments designed by Thomas Bochynek and conducted by Zhuo Chen, Zach Germain and Emmeline Arter were first ran. The experiments were conducted in a 750 ml glass beaker, with 200 g of a soil and water mixture. The calculation for percent moisture (P_w) can be expressed by

$$P_w = \frac{100 * w}{w + s} \tag{1}$$

where w and s are the mass of water and soil, in grams, respectively. The soil and water were mixed separately in a bowel with an electronic hand mixer until the mixture was smooth and homogeneous. Then, the mixture was placed into a beaker in several batches. After 100g and at the end, the soil mixture was lightly patted down with a flat object, like the bottom of a water bottle, so that each layer was flat and smooth. Afterwards, a thin layer of Polytetrafluoroethylene (PTFE) was placed around the inside of the beaker directly above the mud mixture, with a small cotton ball(see figure 2.3. The PTFE layer was dried using a blow dryer until it became dry and visible. The PTFE layer was added to prevent the ants from crawling out of the beaker. PTFE was chosen instead of baby power because baby power could not stay on the sides of the beaker as well as PTFE. Finally, 1.25g of ants were weighed out and placed into the beaker. From past measurements, this equates to roughly $1,562$

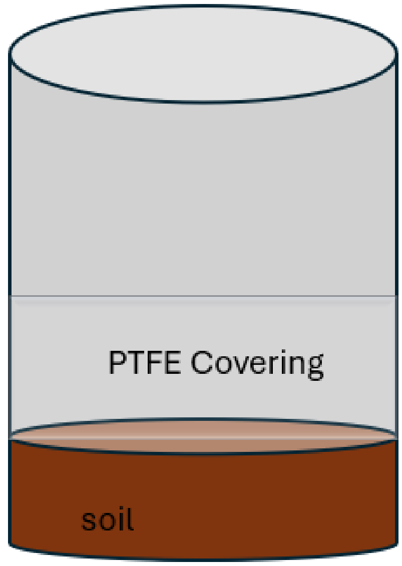

Figure 2.3: Cartoon illustration of beaker set up. In a 750 ml beaker, the 200 grams of premixed soil and water was placed into the beaker and gently patted down to create a quasi-flat surface. A thin layer, with a width of roughly 1 inch, of Polytetrafluoroethylene (PTFE) was then placed on the inside of the beaker walls, directly above the soil layer, via a small cotton ball. The wet PTFE was blow dried until the PTFE layer was no longer wet and a thin white layer of was revealed.

ants; 50 ants were roughly 0.04g. This conversion was done by picking and measuring the mass of 50 random ants (excluding queens) ten times and taking the average of the ten trials. The average of the the ten trails was 0.04g with a standard deviation of 0.02g. For this study, only worker ants of the same colony (c29) were used for the experiments as previous studies have found that surface construction may change in the presence of queens [5]. The beaker was then placed into a climate chamber at 22° C. The sample was retrieved after 24 hours and placed under a heat lamp for another 72 hours, or until the ants are no longer active. The temperature under the heat lamp was not measured. The heat lamp was used to both dry the sample as well as to inactivate the ants. From past observations, the size of ant pellets do not undergo significant change in sizes as a result of this step.

Figure 2.4: Example of clusters of ant pellets. Here, clusters of what appears to be multiple pellets were conglomerated together. These may be fused after depositions of pellets, and were therefore excluded from our analysis. Further investigations will explore the origin of these clusters to assess whether they should be included in analysis.

2.2.2 Pellet size imaging

To image the various ant pellets, first, the pellets were collected by simply gathering the loose pellets from the ant experiment after it has been left in the heat lamp for 72 hours. The loose pellets were collected by tipping the beaker over a fine mesh sieve of 425 microns, the same sieve used to create the soil. This was to separate any loose soil that may have gotten mixed with the pellets. We considered anything larger than 425 microns to be ant excavated and transported. After pellets were sieved twice, tweezers were used to remove non-pellets such as dead ants, small grass or PTFE. Tweezers were also used to remove any clusters of pellets stuck together; only individual pellets are to be imaged (see figure 2.4).We defined clusters of pellets as objects hypothesized to be fused depositions of pellets.

Pellets that were broken were removed from the sample. A flow chart for decision making can be found in figure 2.5.

After sorting out the single pellets, they were placed one-by-one onto the acrylic tablet to be imaged. Pellets were placed onto the acrylic tablet one by one to make sure there were no tangent pellets. At maximum brightness, the background light

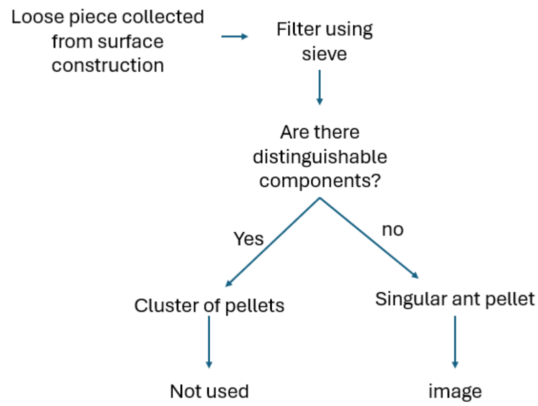

Figure 2.5: A flow chart for determining what to image. First, surface construction is taken from the ant experiment and filtered through a 425 micron sieve. Everything larger than 425 microns was kept and examined to see if it was a single pellet or not. Only single pellets were imaged.

from the light board provided a strong contrast with the ant pellets, allowing for better imaging.

After the pellets have been imaged, they were saved in a crystallizing dish for future references.

Chapter 3

Results, Limitations, and Future Directions

3.1 Results

For this study, six ant experiments, three 20% soil moisture and three 30% soil moisture, were conducted. We compared the area, major and minor axes, and eccentricity of ant pellets collected from 20% and 30% moisture soil. The data collected offered insight into the size and shape of pellets across the two conditions; however, the reason for such distribution is still unknown and can only be speculated (see Future Directions).

3.1.1 Area

As shown in figure 3.1, it appears that at 20% soil moisture levels, there was a broader distribution of pellet sizes. However, the median area of the pellets in the two conditions were surprisingly similar: the median pellet sizes were 0.32mm^2 and 0.36mm^2 for 20% and 30%, respectively. Nonetheless, the differences in distribution suggests that soil moisture in natural soil does affect pellet sizes, which contradict

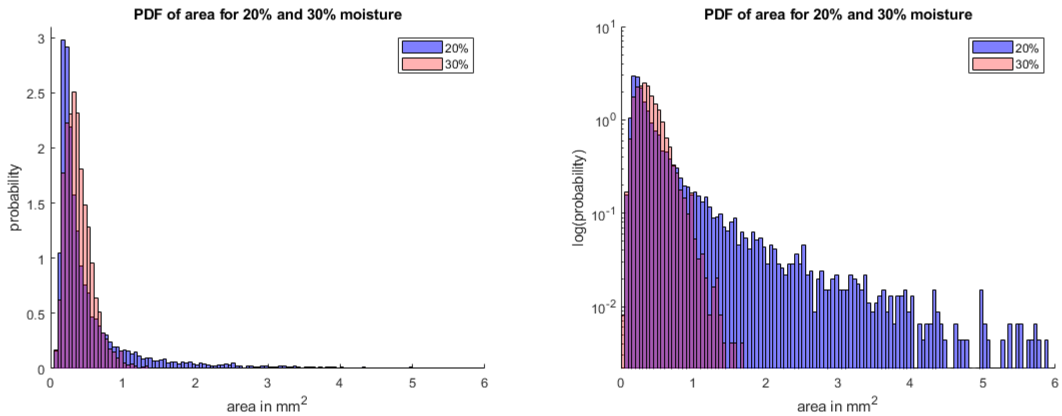

Figure 3.1: Probability distribution functions of areas for 20% and 30% moisture. The distribution on the right has a logarithmic probability to emphasis the tail in the 20% distribution. The two conditions are represented by blue and red, respectively, and the overlap of the two distributions is emphasised in purple. The bin width is 0.05mm^2 for both graphs

previous findings in artificial soil [8]. To learn more about this situation, we also measured the maximum and minimum Feret diameters of each pellet.

3.1.2 Axes

Figures 3.2 and 3.3 revealed that the maximum Feret diameter remained relatively consistent across both moisture conditions. The average maximum Feret diameter was 1.0 mm with a standard deviation of 0.5mm for the 20% moisture. The average minimum Feret diameter was 0.7mm with a standard deviation of 0.4mm for the 20% condition. For the 30% condition, the average maximum Feret diameter was 1.0mm with a standard deviation of 0.5mm and the average minimum Feret diameter was 0.7mm with a standard deviation of 0.2mm. The difference in the broader distribution of minimum Feret diameter measurements in the 20% moisture condition could be a potential reason for the difference in distribution in the area data. After all, the area is quadratically related to the Feret diameters.

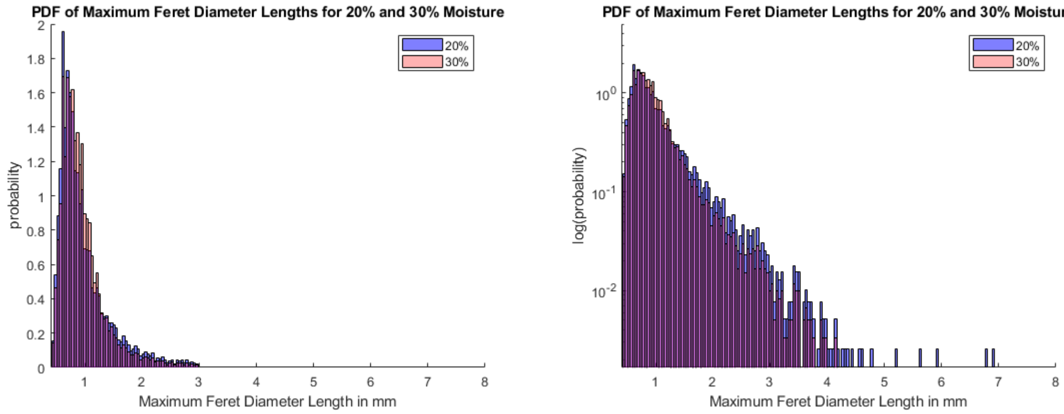

Figure 3.2: A probability distribution functions of the maximum Feret diameters for 20% and 30% moisture. The distribution of the right has a logarithmic probability to reveal the small tail of values in the 20% condition. The two conditions are represented by blue and red, respectively, and the overlap of the two distributions is emphasised in purple. The bin width is 0.0425mm for both graphs

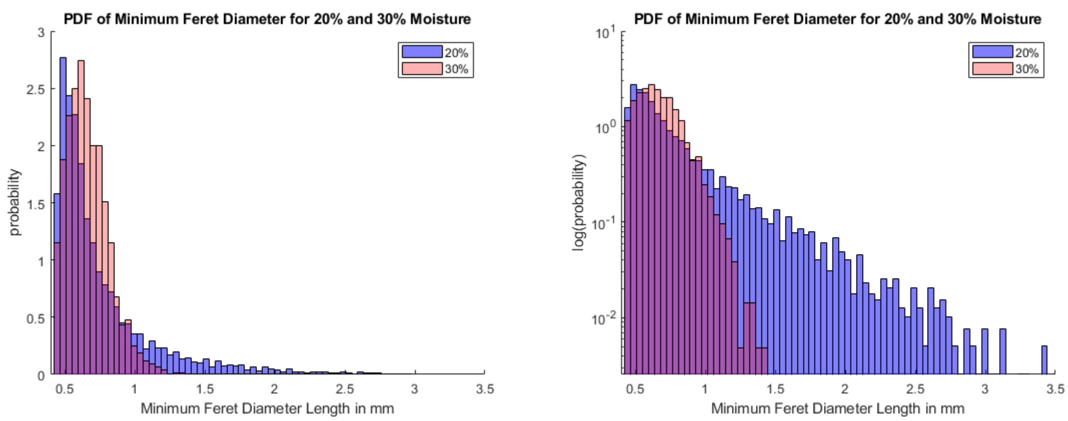

Figure 3.3: A probability distribution functions of the minimum Feret diameters for 20% and 30% moisture. The distribution of the right has a logarithmic probability to emphasis the small tail of values in the 20% condition. The two conditions are represented by blue and red, respectively, and the overlap of the two distributions is emphasised in purple. The bin width is 0.0425mm for both graphs

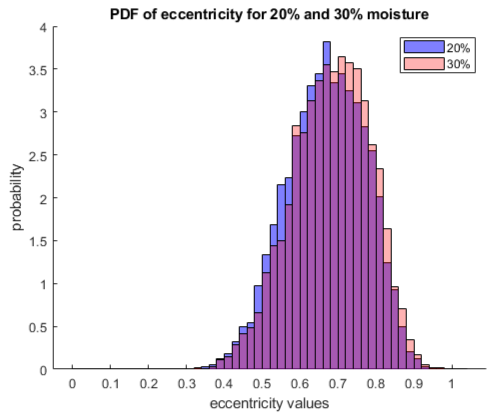

Figure 3.4: A probability distribution function of eccentricity values for 20% and 30% moisture. The two conditions are represented by blue and red, respectively, and the overlap of the two distributions is emphasised in purple. The bin width is 0.02

3.1.3 Eccentricity

In addition to size, we were also interested in the shape of ant pellets across conditions. From figure 3.4, we can see that the eccentricity distribution between the the two conditions were quite similar. The average eccentricity value for the 20% condition was 0.7 with a standard deviation of 0.1 and the average eccentricity value for the 30% condition was 0.7 with a standard deviation of 0.1. The eccentricity values suggest that on average, pellets across both conditions were moderately round.

3.2 Limitations

The first limitation to this study was the fact that the images are all in 2D, but pellets are 3D objects. As a result, we were losing information about another axis, which could have presented data that could better support the results found.

Another limitation was the inherent bias in the definition for a pellet. Many of the loose surface construction in both conditions consisted of clusters of pellets. Due to our definition of a pellet, there was an inherent bias for selecting smaller surface construction from the both conditions, but especially from the 30% condition, which

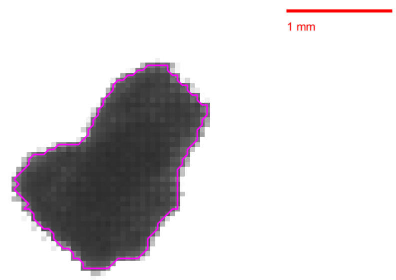

Figure 3.5: Calculated perimeter of a pellet outlined in magenta. From the image, it is clear that not all pixels are considered when Matlab calculates the perimeter of a pellet. This negligence of certain pixels would decrease the the pellet's perimeter, leading to a larger perimeter circularity value. Further tests are needed to verify this hypothesis of error.

tended to contain mostly clusters of pellets. As a result, the findings were probably an underestimate of the true pellet size. However, the findings were relatively consistent with the proposed sizes of ant pellets made by harvester ants, although that study utilized artificial soil instead of natural soil [4].

Finally, there might have been errors related to pixel resolution, which would most likely occur when measuring smaller or jagged pellets. For example, in figure 3.5, in small or jagged pellets, the pixel's location may not be 100% accurate, which can cause a difference in area measurements. Future work will explore the significance of these potential errors through the systematic manipulation and characterization of fake pellet data. Experimentally, these errors could be improved through a better resolution camera or lens with a larger focal length. This would allow for better resolute images for smaller or jagged pellets.

3.3 Future Directions

The findings of this study demonstrated that despite stark differences in surface construction due to soil moisture differences, surface construction pellets remain relatively similar to one another. This suggest that there must be another governing factor that caused the difference in construction. Perhaps soil moisture does not directly affect the shape of pellets but rather the cohesive forces between them which may or may not affect the shape of the pellet.

While additional factors connecting soil moisture and pellet size remain unknown, preliminary experiments using a robotic arm to probe natural soil at different soil moisture content revealed that the penetration force differed greatly between 20% and 30% (see figures 3.6 and 3.7). The 20% had a stronger resistance to penetration compared the 30% condition. This difference in force may explain the larger distribution of pellet sizes in the 20% condition, as compared to the 30% condition.

Additional studies should consider finding a way to image the pellets in 3D to get a better understanding of the pellet's characterises or increase the number of pixels per millimeter in 2D imaging. 3D imaging could be done via a Camsizer, which is a specialized machine that uses a laser to measure small particles' 3D shape. This would allow for a more accurate analysis on a pellet's shape.

Figure 3.6: Pictures of preliminary probe tests on 20% (left), 25% (middle) and 30%(right) moisture soils. Each sample was made by mixing 800 grams of natural soil and water in the exact way as ant experiments, but in spring-form pans. The entire spring-form pan was filled up with the soil and water mixture (about 5.1 cm) before being probed by a thin (diameter of 0.125 inches) aluminum rod attached to a force sensor on a robotic arm. The spring-form pan's edges were taken off during the probe. The probe is then carefully guided slowly into the sample perpendicularly while taking force measurements along the way. The each probe went 4.1 cm into each sample. 0.125 inches of space was left between the bottom of the spring-form pan and the probe to avoid wall effects. Each sample was probed 16 times.

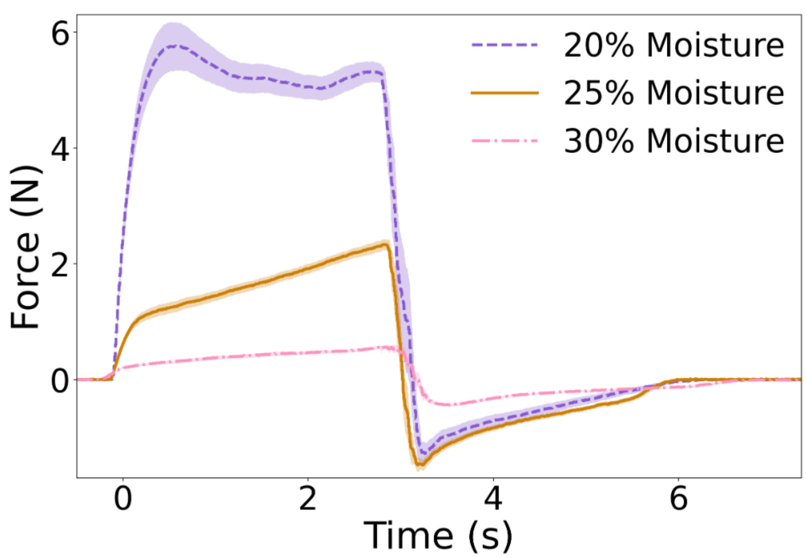

Figure 3.7: Average force over time measured during a robotic probing of soil in three different conditions. (see figure 3.6). There were 3 samples per condition and 16 measurements were made per sample.It was noted that the 20% sample felt more brittle than the 30% condition, which is why there was a slight dip in force as time increased. The shaded regions around each force line represents a 95% confidence interval.

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