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A Comparison of Highly Disturbed Agricultural Soil and Natural Forested Soil as it Affects Decomposition

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by

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This thesis has been examined and approved by the following members of the student's committee.

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List of Abbreviations

SOM – Soil organic matter

PMI – Post-mortem interval

CDI – Cadaver decomposition island

ADD – Accumulated degree days

DON – Dissolved organic nitrogen

DOC – Dissolved organic carbon

Glossary

Anthroturbation – Describes any ways in which humans mix soils

Alfisol – Moderately leached soils that have a relatively high native fertility

Adipocere – Substance formed during decomposition from the fat and soft tissue of the deceased animal

Alluvial – Deposits of soils from running water in a stream or floodplain

Argillic – A subsoil horizon defined by the accumulation of clay transported through alluvial processes

Bioturbation – In soils, the mixing of sediments by living organisms

Colluvial – Deposits of soils from higher areas to lower areas by gravity

Consistence – The degree and kind of cohesion and adhesion that soil exhibits and/or the resistance of soil to deformation or rupture under an applied stress. Consistence is measured by applying pressure to a soil sample and gauging how much force is necessary to rupture (break) the soil unit.

Diagenesis – The change of sediments to sedimentary rock from both chemical and physical changes

Hand texture – Roughly estimating the particle size of a soil based on the feel of a sample

Humus – The dark, organic portions of soil, formed from the decomposition of plants and animals

Mollisol – Soils with a dark colored surface horizon, indicating high organic content

Pedology – Soil science, or the scientific branch that studies soil formation

Pedon – The smallest unit or volume of a soil that contains all of the horizons in order from top to bottom without disrupting the soil's natural structure

Pedoturbation – Any process that mixes soil horizons

Redoximorphic – Reduction of oxygen in soil due to changes in water levels and iron content; leads to reddish stains in the soil

Soil horizons – The sections of a soil formed from a combination of climate, organisms, relief, parent material, and time; each layer has different properties based on depth and these factors

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ANTHROPOLOGY

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ABSTRACT

The purpose of this study is to compare decomposition of animal samples in plowed and unplowed soils. To accomplish this, two sets of three pig (*Sus domesticus*) ham hocks were buried in soil in early May of 2023, one set in a highly disturbed agricultural field and the other set in an undisturbed forested area. Soybeans were planted in the agricultural field within a week. The samples remained buried until excavated in October 2023 after harvest to compare the extent of decomposition and any other changes that occurred to the surrounding soil. Additionally, the soil at each site was described following standard methods of the National Soil Survey Center to determine the factors acting upon the soils. Soil samples from each were analyzed by the EARTH Systems Laboratory at Minnesota State University, Mankato for particle size distribution. Soil samples from pre- and post-decomposition were also sent to Ward Laboratories Inc. of Kearney, Nebraska for chemical analysis. The study found that the ham hocks had all reached similar levels of decomposition and were completely, or nearly completely, skeletonized. More adipocere tissue was recovered on the remains buried in the forest while more invertebrate activity was observed on the remains buried in the plowed field. Chemically, pH decreased in the plowed soil from May to October while pH increased in the forested soil. Nitrate concentration in the plowed soil increased significantly while nitrate concentration decreased in the forest soil from May to October. Due to the high level of decomposition, previous methods for cataloging animal decomposition were not very effective. Future research could include shorter periods of time for analysis, expanding the sample size, and testing over more soil types and treatments.

Chapter 1. Introduction

The purpose of this study is to examine how different soils affect mammal decomposition in a temperate climate. Past studies have looked at decomposition processes in different environments (Carter, Yellowlees, and Tibbett 2007; Megyesi, Nawrocki, and Haskell 2005; Wescott 2018) and how decomposition impacts soil chemistry (Meyer, Anderson, and Carter 2013; Aitkenhead-Peterson et al. 2012; Fancher et al. 2017; Tibbett and Carter 2009; Carter and Tibbett 2006), but few studies have examined decomposition from the perspective of soil formation. Some research has looked at the effects changes in soil type have on decomposition (Tumer et al. 2013; Parson 2019), but soil formation is more than the isolated soil type (clay, sand, or silt). Rather, soil is created from multiple interacting processes which impact decomposing material and affect how the soil interacts with decomposing material.

Soil formation and decomposition are both strongly affected by climate (Schaetzl and Anderson 2005; Byers 2017). For example, temperature affects the amount of water which can enter the soil, with colder soils allowing less infiltration (Schaetzl and Anderson 2005). Moisture, specifically water flow, is also a key factor in transportation of solids and ions within soils. Soils with high clay and organic matter content have better water retention and fine-grained soils are more likely to hold water longer than coarse-grained soils. Additionally, soil temperature is important to plant growth and development (Bakshi and Varma 2011) which in turn can help stabilize soil (Schaetzl and Anderson 2005). Moisture and temperature are also key to soil formation, with soils in warm and/or humid environments showing more development (e.g., Oxisols and Ultisols) than soils in dry environments (e.g., Aridisols) and very cold environments (e.g., Gelisols). From a decomposition perspective, continuously waterlogged, anaerobic soils promote organic material preservation (Kibblewhite, Tóth, and Hermann 2015) as anaerobic organisms, which are less efficient at decomposition, are more prominent in these environments (Carter, Yellowlees, and Tibbett 2007). Cooler temperatures also slow microbial activity, additionally preserving remains (Byers 2017; Carter and Tibbett 2006), while hotter and more humid climates speed up decomposition (Perper 2006; Emmons et al. 2022; Byers 2017). These are just some examples where soil formation and animal decomposition processes overlap. Therefore, studying decomposition from the perspective of how a soil was formed can in turn improve the guidelines for predicting postmortem intervals (PMI) of a decedent individual.

For this study, six cuts of pig (*Sus scrofa domesticus*) legs (i.e., ham hocks) were buried in similar soils with different vegetation coverage and allowed to decompose during the summer of 2023 in Minnesota. The soils were formed following the same glacial period, on the same landform, at similar elevations, and were exposed to similar levels of moisture. One set of samples was buried in a plowed, agricultural field with soybean coverage while another set of samples was buried in a deciduous forested area. The sites were mapped and excavated after harvest, and once cleaned, the decomposition levels were compared. The temperature and average rainfall data from the study period were downloaded from the Minnesota Department of Natural Resources and the accumulated degree days (ADD) were calculated at the end of the experiment. Soil samples from before and after decomposition site were sent to Ward laboratories for chemical analysis and another set of soil samples were sent to the EARTH Systems Laboratory for particle size analysis.

This study provides novel data about decomposition from a soil forming perspective to the literature. Climate, soil structure, and the chemicals within the soils are considered when discussing the decomposition results. Additionally, the effect decomposition has on the surrounding soil is considered and discussed.

Chapter 2. Literature Review

2.1 Understanding decomposition

Decomposition is the process of rotting, or the breakdown of materials into simpler compounds. For the purpose of this study, decomposition is the breakdown of organic materials into inorganic base materials. Decomposition of human remains, specifically understanding the timeframe and process, is important to the field of forensic anthropology (Perper 2006). By understanding the sequential order of changes to remains that occur postmortem, scientists can create a timeframe of events including time of death and if the remains were moved or altered postmortem. Previous research has looked at how burial depth affects decomposition and recovery (Rodriguez and Bass 1985), how physical disturbance can impact soft tissue decomposition (Adlam and Simmons 2007), and how temperature changes the rate of microbial decomposition (Carter and Tibbett 2006). Much has also been written about the decomposition process in general (Byers 2017; Astolphi et al. 2019; Pollock, Pokines, and Bethard 2018; Emmons et al. 2022; Perper 2006). At Minnesota State University, Mankato, there has been research on how decomposition is affected by the winter months in Minnesota (Bartlett 2015; Herbes 2023). While many studies have explored how decomposition impacts the soil (Fancher et al. 2017; Aitkenhead-Peterson et al. 2012; Carter, Yellowlees, and Tibbett 2007), few, if any, have looked at decomposition from a soils perspective. By applying the factors that affect soils to the discussion, the decomposition process can be better understood as a whole.

2.2 Stages of decomposition

Decomposition can be broken down into stages: fresh, active decomposition, advanced decomposition, and skeletonization (Tibett and Carter 2009; Emmons et al. 2022; Perper 2006; Stuart 2013; Megyesi, Nawrocki, and Haskell 2005). Few macroscopic changes are observed during the fresh stage of decomposition, but factors like temperature and location of the remains can speed up or slow down the process (Byers 2017; Stuart 2013). Internally, cells will continue to function until oxygen is depleted (Emmons et al. 2022). Eventually, cellular function will break down, cellular membranes will weaken, and organelles will be released into intracellular space. This process begins autolysis, or the breakdown of cells internally, also known as "self-digestion."

Despite autolysis beginning in fresh decomposition, macroscopic changes may not yet be observable (Emmons et al. 2022). Not all cells degrade at the same rate, with cells in the gastrointestinal system likely to break down first while connective tissue breaks down last. Microorganisms from the gut, skin, and mouth – particularly gut bacteria – are able to reproduce unchecked and decompose the body internally (Emmons et al. 2022; Byers 2017). This process, known as putrefaction, occurs simultaneously with autolysis. Because both processes occur in conjunction and feed into each other, it is difficult to separate individual phenomena during this stage of decomposition.

Externally, Diptera (fly) activity can begin within minutes of death, and subsequent activity has been used to estimate the postmortem interval (PMI) (Singh et al. 2019; Megyesi, Nawrocki, and Haskell 2005; Byers 2017). The eyes are usually the first part of the corpse that show death, sometimes developing a sheen within a few minutes and becoming cloudy within a few hours (Byers 2017; Emmons et al. 2022). An onset of pallor may affect the corpse, making the skin appear whiter, and the contents of the bowels may be released due to muscle relaxation. Three mortises occur within one to four hours after death and can occur earlier (Emmons et al. 2022). Livor mortis is when the blood pools in the direction of gravity and leaves bruise-like marks. Rigor mortis takes place over a span of around 48 hours, but can range from 24 to 48 hours after death. During this process, the muscles in the body stiffen and eventually release, though rigor does not occur simultaneously throughout the body. Algor mortis is the shift in body temperature to match the surrounding environment and typically occurs within 18 to 20 hours.

Early decomposition, or active decay, can begin within 24 hours of death (Emmons et al. 2022; Byers 2017). Autolysis begins the process of early decomposition and is followed by putrefaction causing discoloration of the remains (Emmons et al. 2022). Putrefaction also causes bloating and odors, while autolysis leads to skin slippage, hair loss, and fluid-filled blisters. Pressure builds in the abdomen, face, and scrotum (in males), eventually leading to the rupture of materials from the eyes, nose, mouth, and anus. During this entire process, insects – particularly flies and their larvae – will continue to be attracted to and feed on the remains (Byers 2017). Insects will target areas with the most direct access to the interior of the remains, including natural openings like the mouth, anus, eyes, etc. and any cuts sustained peri- or postmortem (Byers 2017; Campobasso, Di Vella, and Introna 2001). The combination of internal pressure and predation from insects will cause the abdominal cavity to rupture and further release the decomposition fluids into the surrounding soil (Emmons et al. 2022). The decomposition byproducts released into the soil create an area known as a *cadaver decomposition island* (CDI), or an area with elevated soil microbial communities (Emmons et al. 2022; Aitkenhead-Peterson et al. 2012; Carter, Yellowlees, and Tibbett 2007).

Advanced decomposition begins just prior to larval migration, near the end of active decomposition (Emmons et al. 2022). The remains will begin to look deflated and wrinkled in response to mass loss caused by maggot and other insect activity. While skeletal exposure may begin at this stage, especially in areas with little soft tissue like the skull, the remains will still appear moist and fleshy elsewhere. A rich CDI is also characteristic of this stage, with the surrounding soil being inundated with high nitrogen, carbon, and other nutrients like magnesium, calcium, potassium, and phosphorus (Emmons et al. 2022; Aitkenhead-Peterson et al. 2012). At this stage, Diptera (fly taxa) are no longer the predominant insect involved in decomposition but are replaced by *Coleptera* (beetle taxa).

2.3 Preservation

During active and advanced decomposition, the remains can begin to dry out, depending on the surrounding environment (Emmons et al. 2022; Byers 2017). Mummification, rather than skeletonization, can therefore be the end result of decomposition, and can occur in both extreme heat and extreme cold. Another form of preservation that does not lead to skeletonization is adipocere formation (Emmons et al. 2022; Byers 2017; Carter, Yellowlees, and Tibbett 2007; Wescott 2018; Perper 2006). Many mammals have sufficient fat and moisture to form adipocere, or “grave wax,” which can appear greasy, hard, or soft, as a waxy gray/white material, or as saponified fat (Emmons et al. 2022). Adipocere is the result of free fatty acids hydrogenating to saturated fats, with the appearance and texture dependent on the surrounding environment (Emmons et al. 2022; Perper 2006; O'Brien and Kuehner 2007; Tsokos and Byard 2016). Sodium additions cause a hard "crumbly" texture while potassium stimulates a soft "paste-like" texture (Emmons et al. 2002). Adipocere will also harden and crumble as fatty acids crystalize overtime (Tsokos and Byard 2016). Adipocere formation is typically associated with wet environments, but the necessary variables are adipose (fat) tissue, bacteria, anaerobic conditions, and moisture, meaning the moisture from the corpse can also be adequate for formation, especially if the remains are in an enclosed, non-permeable environment. *Clostridium perfringens* is the most prevalent bacteria involved in the formation of fatty acids, but other species are involved as well. Mildly alkaline pH conditions also encourage adipocere formation (Ubelaker 2023). Adipocere was previously believed to only form in standing water, but it is now understood that the remains alone can contain enough moisture to allow for adipocere formation (Tsokos and Byard 2016). Adipocere does preserve the remains, especially the portions with the most fatty tissue, by preventing skeletonization. But adipocere can also decompose if moved from a burial to the surface or if introduced to an aerobic environment (Carter, Yellowlees, and Tibbett 2007).

2.4 Skeletonization and bone degradation

Skeletonization is reached when more than 50% of the skeleton is exposed (Emmons et al. 2022; Byers 2017; Megyesi, Nawrocki, and Haskell 2005). While the underlying soil may not return to previous conditions, the surrounding environment generally reverts back to its pre-decomposition state (Emmons et al. 2022). Skeletonization is often referred to as the final stage of decomposition (Emmons et al. 2022; Byers 2017; Megyesi, Nawrocki, and Haskell 2005; Carter, Yellowlees, and Tibbett 2007), but bone continues to interact with the environment and can be further degraded or built up depending on the chemical surroundings (Emmons et al. 2022; Astolphi et al. 2019; Booth 2017; Bell, Skinner, and Jones 1996; Stuart 2003).

Any alterations to bone are known as diagenesis (Booth 2017; Emmons et al. 2022). During diagenesis, bone can be infiltrated by external forces and cause alterations to the bone by altering the biological or mineral components of the bone. Microbial bioerosion, or the biological degradation of the mineral and organic portions of the bone, is the most common form of diagenesis, and is often seen in archeological bones from temperate environments. During this process, bacteria use the natural holes in bones, like the Haversian canals, to eat away at the bone and create a wider system. This, in turn, makes the bone feel lighter and more fragile than fresh bone. Simultaneously, "bone petrification" occurs (Astolphi et al. 2019). This is where the mineral portion of the bone matrix, in absence of the organic portion, takes on the features of the surrounding environment, including iron oxides, carbonates, or sulfides, and leaves the rigid part of the bone. While this does not happen with every decomposition, the remains are essentially fossilized and therefore better preserved (Astolphi et al. 2019; Emmons et al. 2022). Biological degradation in bone continues until the collagen is completely destroyed and nitrogen can no longer be derived (Kibblewhite, Tóth, and Hermann 2015).

Unless preserved either naturally or by human intervention, bone remains will continue to degrade from their exposure to the environment (Bell, Skinner, and Jones

1996). Bones exposed to sun and wind go through a process known as "weathering" (Perper 2006). Weathering will bleach the bones and cracks will form from being exposed to the sun and wind (Byers 2017). The sun and wind strip down the cortical surfaces of the bone and cause flaking of the denser exterior surface, a process that makes the bones lighter and more fragile. If the bones are not covered, natural events like rock falls can further damage the bones. Similarly, if the bones are in moving water, they can be separated and further damaged from scraping against hard surfaces. If the remains are burned for any reason, the bones, like the flesh, can be further decimated, though complete dissipation of a skeleton from a fire is unlikely (Castillo et al. 2013). Heat breaks down the size and shape of the bone, and even if not fully destroyed in a fire, can severely weaken the structure, making further breakage more likely.

2.5 External factors that impact decomposition

While decomposition has been studied for nearly 40 years (Rodriguez and Bass 1985; Megyesi, Nawrocki, and Haskell 2005), the decomposition process is difficult to sequence because many external factors can speed up or slow down the process (Emmons et al. 2022; Byers 2017; Perper 2006; Megyesi, Nawrocki, and Haskell 2005). Important factors in decomposition are temperature, humidity, and accessibility, with temperature being the most important because this also affects plant, animal, and microbial activity (Byers 2017; Emmons et al. 2022; Wescott 2018; Campobasso, Di Vella, and Introna 2001). Warmer temperatures increase plant growth, animal and insect scavenging, and bacteria duplication while colder temperatures encourage the opposite. As a general rule, for every 10°C increase in temperature, enzymatic activity increases by a factor of two or three (Emmons et al. 2022; Wescott 2018; Meyer, Anderson, and Carter 2013). Therefore, temperature exposure can be used to further narrow down the PMI using accumulated degree-days (ADD) to calculate the energy available to the remains (Megyesi, Nawrocki, and Haskell 2005) and can be used to predict how much decomposition has occurred during the PMI (Michaud and Moreau 2011). A regression model using the ADD can be used to predict how much decomposition has occurred, where Y is equal to the accumulation of degree days above 6, 10, 12, and 13°C (Michaud and Moreau 2011). When Y=1.75, the onset of skeletonization is predicted. Plant growth is also slowed during colder months, and while many animals scavenge in the winter, frozen remains and remains buried in snow are not as accessible (Bunch 2009).

Humidity is the second most important factor that impacts decomposition because the remains are prevented from drying out when humidity is high (Byers 2017; Campobasso, Di Vella, and Introna 2001). Dry remains are less attractive to predators and can ultimately preserve the flesh and organs through mummification. Hot and humid climates are therefore known for being the most efficient areas for decomposition (Schaetzl and Anderson 2005), with one known case of near-total skeletonization occurring in 10 days during a relatively hot and humid summer in Mississippi (Perper 2006).

Accessibility is the third most important factor to decomposition as this determines which animals and insects will be able to aid in decomposition, and any inhibition of access slows decomposition (Byers 2017; Emmons et al. 2022). As mentioned, fly and beetle varieties are the predominant species that aid in decomposition. Because the life cycle and interactions with decomposition are so scheduled, the presence of flies and beetles have been used for estimating PMI (Emmons et al. 2022; Wescott 2018; Campobasso, Di Vella, and Introna 2001; Singh et al. 2019). Insects are so efficient at decomposition, they can increase the percentage of body mass lost by 60% over a period of five days when compared to carcasses with no insect interference (Emmons et al. 2022). While insects are largely associated with early decomposition, some insect-like invertebrate species (springtails and mites [Family Entomobryidae and Glycyphagidae, respectively]) have been found on remains interred for 28 years (Merritt et al. 2007). Larger species including rats, coyotes, and bears are known to scatter remains, leading to many decomposition studies relying on protective barriers (cages, ties, etc.) to prevent the loss of the specimen (Byers 2017; Herbes 2023).

Accessibility includes if the remains are on the surface, in water, or buried (Byers 2017; Emmons et al. 2022; Rodriguez and Bass 1985; Tibbett and Carter 2009; Wescott 2018). Remains that are exposed to air will decompose faster than those in water, which in turn is faster than decomposition in soil (Perper 2006; Byers 2017). For a general timeline of decomposition, one week exposed to the air is equal to two weeks in water and eight weeks in soil. While water runoff from precipitation has not been shown to impact decomposition (Byers 2017), standing water and water-saturated soil does limit insect and microbial activity, slowing decomposition (Emmons et al. 2022; Byers 2017; Tibbett and Carter 2009; Booth2017; Kibblewhite, Tóth, and Hermann 2015).

Like submerging remains in water, burial depth inhibits certain insect and microbial activity (Campobasso, Di Vella, and Introna 2001; Rodrigues and Bass 1985; Kibblewhite, Tóth, and Hermann 2015; Emmons et al. 2022; Carter, Yellowlees, and Tibbett 2007) and therefore, organic activities, including decomposition, decrease with soil depth (Kibblewhite, Tóth, and Hermann 2015; Schaetzl and Anderson 2005; Bakshi and Varma 2011). Carrion insects are only observed to depths of 30 cm (11.8 in), further slowing a primary decomposition driver (Rodriguez and Bass 1985). Similarly, biological activity of soil is largely concentrated to the topsoil, varying from a few centimeters to ~30cm in depth (Das and Varma 2011; Schaetzl and Anderson 2005). Microorganisms in soil include eubacteria, fungi, algae, protists, and viruses (Bakshi and Varma 2011). The amount and type of microbes that saturate the soil not only depend on depth, but also the type of soil and moisture levels.

2.6 Soil particulates and properties

The primary soil particles are sand, silt, and clay, which make up the inorganic fractions of the soil (Schaetzl and Anderson 2005; Al-Kaisi et al. 2017). The distribution of sand, silt, and clay in a sample determines its texture (Schaetzl and Anderson 2005; Schoeneberger et al. 2011). These divisions are based on particle size, with sand being the coarsest, clay being the smallest, and silt being intermediate sized (Schaetzl and Anderson 2005). In nature, these fractions are typically mixed forming variations like sandy clay, or silty loam, with loam being a near equal mixture of all three. The size and shape of the inorganic matrix also influences the chemical, physical, and biological properties of the soil.

Particle size distribution as well as aggregation of the individual particles to form structure influences the pore size, or empty space, of the soil (Al-Kaisi et al. 2017). Water is both adsorbed (on the surface of soil particles) and absorbed (soaked into the aggregates) into soils, depending on the soil matrix, with clay and soil organic matter serving as better retainers of moisture (Schaetzl and Anderson 2005). Some clay minerals swell when wet or can be displaced into pores between soils, slowing and even inhibiting water flow. Additionally, clay aggregates have high internal porosity, but pores are poorly connected, inhibiting water movement (Schaetzl and Anderson 2005). Clay has a larger surface area than sand or silt, increasing its ability to adhere to moisture and organic matter in the soil matrix. Sandy soils with large macropores (space between grains) are better conductors of water than fine-textured soils (Schaetzl and Anderson 2005; Al-Kaisi et al. 2017). Working in conjunction with particle size is the amount of moisture already present in the soil. When soils are dry, water flows from saturated areas to unsaturated areas, but if the soil is already inundated with water, gravity will pull water further into the ground. Distinct changes in particle size can also cause water to 'perch' between soil horizons, or soil layers. If a sandy (coarse-textured) soil overlays a clayey (fine-textured) soil, water flow from the top layer to the lower layer with less hydraulic conductivity may be inhibited until the fine-textured soil absorbs the moisture. Perching can also occur when fine soils overlay porous soils if the water pressure is insufficient to displace the air held in the larger pores of the coarse-grained soil. Typically, water will remain in the fine-grained soil until saturated, or nearly saturated, before the pressure is enough to flow into the coarse-grained soil. Flow paths can then be established, creating a preferred area for water accumulation.

Soil temperature additionally affects water and gas flow through the soil. For example, colder air temperatures can cause soil frost which makes the soil less permeable and leads to erosion and runoff (Schaetzl and Anderson 2005). Multiple factors affect soil temperature including moisture content, heat capacity, ground cover, and fluctuations in air temperature, making predicting soil temperature a complicated endeavour. Generally, water content in soil influences variation in temperature more than mineral, gas, and organic matter as water has a higher heat capacity than the other factors. Coarse-grained soils are therefore better thermal conductors as water can flow more freely through the soil. Additionally, while surface heat does impact deeper soil, there is a lag between surface heat and the temperature beneath the surface. This difference increases with depth, with soils closer to the surface having temperatures that more closely resemble the current air temperature and deeper soils maintaining temperatures from past seasons. For example, the surface of the soil will reach its max temperature in July while the same soil at 500cm will not reach the max temperature until December (Schaetzl and Anderson 2005). Daily oscillations are only measurable to about 20 cm and only vary by approximately 2°C. By 50cm, daily temperature oscillations have been nearly completely diminished.

In animal decomposition, sandy soils with low moisture content are most associated with desiccation, likely due to the soil's ability to move gasses through the soil (Carter, Yellowlees, and Tibbett 2007). These types of soils are also likely to lose moisture and slow down enzyme activity which prefers moist environments (hydrolytic) and is most associated with carbon and nutrient cycling. Fine, clayey soils can also prevent decomposition due to a low rate of hydraulic conductivity which can in turn trap air in soils and decrease oxygen flow (Schaetzl and Anderson 2005). Aerobic microorganisms are unable to get the oxygen necessary to function as the oxygen and carbon exchange rate is low, making anaerobic microorganisms, which are less efficient at decomposition, the dominant microbe present (Carter, Yellowlees, and Tibbett 2007). This can also promote adipocere formation.

2.7 Soil chemistry

Microorganisms in soils are affected by both the natural processes acting on soils and human intervention, specifically agricultural practices. One study in Georgia (USA) comparing cropland soils to forest soils found that the pH, nitrate, phosphorus, and calcium levels were much higher and soil organic matter (SOM) was lower in the cropland soils compared to the forest soils (Wu et al 2023). This study additionally found that agricultural practices had significantly decreased the bacterial richness, abundance, and diversity in the crop soils when compared to the forest soils. More nitrifying bacteria was present in the crop soils while denitrifying bacteria diversity had been diminished. This potentially affects the plant productivity and nitrogen cycling of the soils. Another study in Georgia performed over a decade prior had similar results. Established forest soils were compared to recently forested soils and cropland soils, with lower bacteria diversity, but more abundant taxa in the forest soils than in cropland soils (Upchurch et al. 2008). This study also found that the pH and nitrate levels of the cropland soils were higher than the forest soil. It should be noted, however, that soil pH in the southeastern United States is naturally more acidic than the soil in the Midwest due to higher annual rainfall (Snyder 2014). In the Midwest, cropland soils can become more acidic than their natural state from nitrogen fertilizer additions and crops that take up more cations, like legumes. Liming crop soil is a common practice to counteract the acidification of soil, increasing the pH and crop yields.

The changes in pH and nitrogen levels in soil can also reflect the microbiome community and can help predict the pace and progress of SOM decomposition.

Decomposition is known to affect and be affected by soil chemistry (Rodriguez and Bass 1985; Tibbett and Carter 2009; Fancher et al. 2017; Aitkenhead-Peterson et al. 2012; Schaetzl and Anderson 2005), with noticeable changes in the pH, sulphates, electrical conductivity, nitrogen, and carbon content (Aitkenhead-Peterson et al. 2012). Bacteria and actinomycetes are the main decomposers when the pH is greater than 5.0 while fungi are the main decomposers when the pH is less than 5.0 (Schaetzl and Anderson 2005; Byers 2017). Mull humus is produced in higher pH values from bacterial decomposition while a more greasy humus is the result of a lower pH and fungal decomposition process. Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) are shown to increase directly beneath cadavers when compared to control soils (Fancher et al. 2017; Aitkenhead-Peterson et al. 2012). Because these changes are known, increases in carbon, nitrogen, electrical conductivity, and pH changes in soil surrounding known cadaver decomposition have been studied to better predict the PMI in missing persons cases and when attempting to identify graves (Rodriguez and Bass 1985; Tibbett and Carter 2009; Fancher et al. 2017; Aitkenhead-Peterson et al. 2012).

<u>2.8 Studies involving soil and decomposition</u>

Fancher and colleagues observed soil chemical changes in response to cadaver decomposition in two different soil series (Fancher et al. 2017). Soil samples were taken from the CDI of each cadaver sample, either when the corpse was removed from the area or from the soil under the groin area, to measure concentrations of decomposition-related chemicals including the DON and DOC. Changes in chemical concentrations were observed over a span ranging from 6 to 1,752 days. Measurements of the chemicals in the soils were taken at increments within this timespan. Variance between soil types noted as well. For example, DOC concentration doubled at 6 days postmortem in one soil series and had a 13-fold increase at 33 days in the other. DON also showed a rise in concentrations for both soils but did not return to ambient concentrations within the timeline of the study.

Other studies have looked at the soil chemistry which encourages or inhibits decomposition (Peralta and Wander 2008; Hall, Russell, and Moore 2019; Bonner et al. 2019). One study found that increased carbon dioxide levels (CO_2) reduced carbon and nitrogen stocks in soil, leading to a decrease in soil organic matter (SOM) because the organic matter was decomposing faster (Peralta and Wander 2008). More recent studies have examined how inorganic N can reduce plant litter and SOM decomposition rates (Bonner et al. 2019). Bonner and colleagues (2019) found that high rates of N addition affect the enzyme expression in soil which negatively impacts the breakdown of lignin in plant material and slows decomposition. One hypothesis behind this effect is that inorganic fertilizers are satiating the nitrogen demands in the soil (Mahal et al. 2019). This will cause an increase in SOM, potentially affecting the makeup of the soil overall (Schaetzl and Anderson 2005). Soil organic carbon (SOC), however, is found to increase with soybean and corn crop rotations (Hall, Russell, and Moore 2019). Soils with a low carbon to nitrogen ratio (C:N) (e.g. soybeans) are shown to encourage microbial growth, and can increase decomposition of soil carbon and litter in subsequent crops with a high C:N ratio (e.g. corn).

Other studies have indicated that increasing carbon content in compost may be necessary when decomposing animal, specifically pig, remains (Lim and Zulovich 2018). Adding sawdust, which is high in carbon, to compost is shown to encourage an ideal C:N ratio for decomposition (25:1), though decomposition is possible and compost can be achieved with variations on the ratio. Additionally, the C:N ratio has been shown to widen as organic material, specifically human and pig cadavers, decompose, suggesting the absorption of carbon into the surrounding environment as the material breaks down (Carter and Tibbett 2006).

Some studies have observed how soil type affects decomposition, specifically looking at soil with different particle size (Tumer et al. 2013; Parson 2019; Forbes et al. 2005). Tumer et al. observed decomposition of wild boar (*Sus scrofa*) extremities in four different soil types (loamy, clayey, organic, and sandy), with all the samples buried in graves lined with tulle to separate the soil samples from the natural setting (2013). The samples were allowed to decompose for six months, then tested for chemical, pH, and electrical conductivity changes between control samples and post-decomposition. The organic matter content was found to increase in the loamy, clayey, and organic soils, but did not show a significant increase in the sandy soil. The pH of the loamy and organic soils was shown to decrease and were also shown to have decomposed more than the samples in the sandy and clayey soils. More adipocere was found on the samples in the clayey and loamy soils.

The results of this study contradicted the results of Forbes and colleagues (2005) which found more adipocere formation on samples in loamy sand and silty sand soils.

However, the authors of this study concluded that when other factors are the same, the soil type was secondary to adipocere formation. The study also kept the domestic pig samples in tightly controlled, anaerobic, moist environments, ideal for adipocere formation. All samples in each soil tested therefore showed adipocere formation. A similar study was performed in 2019 (as a part of student research) which observed chicken (*Gallus gallus domesticus*) carcasses in plastic bins filled with different soil types (Parson 2019). For this experiment, sand, topsoil, and a manure/compost mixture were used. The remains decomposed the fastest in the manure/compost mixture, likely due to a combination of moisture retention and bacterial activity, while the sand had the lowest decomposition rate.

Haslam and Tibbett compared decomposition in contrasting pH levels and found that a Podsol soil (acidic; pH 4.6) was the most efficient for decomposition (2009). The other soils in the study, a Cambisol with neutral pH (6.4) and an alkaline Rendzina (pH 7.8), experienced similar levels of decomposition at the end of the study, but decomposition was most rapid in the Podsol soil. All of the soils became more alkaline post decomposition, with the Podsol seeing the most drastic difference in pre and post decomposition pH levels, followed by the Rendzina and the Cambisol.

While all these studies do demonstrate the efficiency of different soil types at decomposition, natural soil processes are at least somewhat inhibited by separating the soil type from their natural environment. Additionally, other than the climate observations made during the studies, climate and soil forming factors were not accounted for in the assessments.

2.9 Soil pedology and classification

While understanding soil chemistry is important for both forensic investigations and agricultural productivity, soil formation has a lasting effect on the soil chemistry and will impact organic decomposition. Soil, for the purpose of this study, is the naturally occurring material on the earth's surface, formed through the interactions of five major factors: climate, organisms, relief, parent material, and time (Schaetzl and Anderson 2005) and is distinct based on these factors (Brady and Weil 2010). Soils are formed from diverse origins and thus show multiple characteristics depending on their origins (Hartemink et al. 2020). This heterogeneity can stem from the soil's parent material (its geologic origin) and other pedologic factors that have acted upon the soil. When describing soils, structure, texture, horizonation, consistence, and color are used to interpret how the soil forming factors have created the current soils and how the soil continues to interact with the surrounding environment.

For example, coarse-grained particles in sediment can be transmitted by surface winds but travel much shorter distances than fine-grained particulates that are typically deposited in more distal downwind areas (Sun et al. 2002). The particle size of the soil can therefore indicate where the parent material of the soil originated and what climatic events lead to its formation. This in turn determines how the soil interacts with the environment. The permeability of glacial till, for example, can vary greatly due to the variance in fraction size from the depositional event (Schaetzl and Anderson 2005). Soil formed from the same glacial deposit can have areas with low permeability due to a high density of fine sediment while other areas can contain larger fractions and have a higher permeability (Clarke 2018). Water retention and available moisture in the soil are affected by particle size and pore distribution, with eroded soils retaining water as the organic top layer of the soil is more likely to be removed (Arriaga and Lowery 2003). The color or the soil is also important to consider as darker soils typically have a higher organic material concentration, though manganese concentrations are also an explanation (Schaetzl and Anderson 2005; Carter, Yellowlees, and Tibbett 2007). In well-drained soils with iron concentrations, red and brown colorization is possible (Dupras and Schultz 2013). The color of the soils can also stain interred bone (Stathopoulou et al. 2019; Dupras and Schultz 2013).

Climate includes the average temperature and precipitation in an area, which have cascading effects on the remaining factors that affect soil development (Schaetzl and Anderson 2005; Brady and Weil 2010). Organisms, including plants, animals, bacteria, and viruses, move the soil and deposit organic matter through the substrate in a process called bioturbation. Relief refers to the location on the landscape the soil is placed, with soils less likely to form on steep slopes than flat plains. The parent material is the base material soils are formed from which in turn determines what type of soil can form. For example, much of the soil in Minnesota is formed from glacial till deposited during the last glaciation (White 2020; Clayton and Moran 1982; Lusardi and Dengler 2017). Time is the most important to soil formation as this determines how long the other factors have to impact the soil. The breakdown of sediment into smaller particles, organic material to accumulate in the soil, moisture permeates throughout, and other processes occur over time (Schaetzl and Anderson 2005; Arriaga and Lowery 2003). However, time can be reset at any point if the built-up horizons are reduced down to the parent material (Schaetzl and Anderson

2005). For example, a river that floods regularly may have stacked C horizons with no organic layers because the time process has been reset.

Horizons are also important when examining decomposition in soils as each horizon has different characteristics which will affect decomposition (Schaetzl and Anderson 2005; Brady and Weil 2010). Specifically, the O, or organic, horizon is formed of decomposing plant matter. When decomposed, the raw organic material, humus*,* can translocate into the A horizon. The additions of the decomposing organic material into the inorganic substrate, making the soil dark, is known as "melanization." The A horizon is traditionally the "topsoil" layer, or the layer that has been in contact with decomposing organic material long enough to form a layer. The A horizon typically has all, or the majority, of its original geologic characteristics altered (Hartemink et al. 2020). If the A horizon has been plowed or artificially disturbed, it is annotated as an Ap horizon.

C horizons are unaltered or slightly altered parent material. B horizons are subsurface soils which can hold accumulations of iron, aluminium, clay, humus, and more, but do not contain as much organic material as the A horizon. B horizons may contain some of the non-altered geologic materials of the C horizon but have been altered to the point of forming a new horizon. Other horizons include the E horizon, which is a mineral horizon that has been leached of much of its organic material, and the R horizon, the bedrock layer (Schoeneberger et al. 2001; Schaetzl and Anderson 2005). Every soil does not have every horizon, though an A horizon is typically at the surface and the C horizon is above the R horizon, if the R is present. When describing soils, the master horizon (O, A, E, B, C) can be followed by a suffix which further describes the contents of the soil. For example, 'k' describes calcium carbonate ($CaCO_3$) concretions while ‘p’ describes an artificial disturbance, usually plowed soil. Numbers are used both as prefixes and suffixes to delineate if the horizons come from the same soil forming factor or if there were discontinuities.

Soils can be classified taxonomically (Brady and Weil 2010). Similar to the classification of biological systems, soils are classified using broad to specific categories which are: order, suborder, great group, subgroup, family, series, and phase. Order, with only twelve divisions, is the broadest category and is largely based on the absence or presence of specific horizons or specific, mineralogical properties, or temperature/moisture extremes. For example, Mollisols are characterized by having a dark, thick surface horizon, with a high base saturation, which has developed under a grassland environment. Alfisols can similarly have a high to medium saturation rate but are mildly acidic and accumulate clay. Both orders are found in Minnesota (USDA Natural Resources Conservation Service, n.d.).

2.10 Anthroturbation in Minnesota

Pedoturbation describes any processes that mix and change the makeup of the soil (Schaetzl and Anderson 2005). Pedoturbation impacts pedogenesis as the process is largely responsible for the microporosity of the soil which in turn affects runoff and the organic matter content of the soil. Anthroturbation describes how humans mix soils. Like other forms of pedoturbation, anthroturbation can change the horizonization of soils. When the surface horizon is mixed by plowing, these soils are described with an Ap horizon, which are common in Minnesota, as around 26 million acres of the state’s land is used for agriculture, including cultivated cropland (Minnesota Board of Water and Soil Resources, n.d.). Ap horizons are characterized by a sharp lower boundary (described as 'abrupt') where the disturbance overlays the naturally occurring soil. Plowing also destroys the structure of the soil and reduces organic matter content, leading to increased runoff and can cause an agric horizon to form below the Ap horizon, where root channels, ped surfaces, and worm holes become coated in the dark, organic silt and clay particles of the overlaying horizon. If the O horizon is absent, the A horizon will have the most organic activity in the soil.

2.11 This study in context

Understanding soil is important to forensic anthropology and archeology because the same factors that impact soil formation affect decomposition and preservation of organic remains (Carter and Tibbett 2006; Byers 2002; Astolphi et al. 2019; Schaetzl and Anderson 2005; Brady and Weil 2010; Bakshi and Varma 2011). Studies have been performed on the relationship between decomposition and soils, looking at insect activity in response to temperature and burial depth (Campobasso, Di Vella, and Introna 2001), with some of the first studies focusing on burial (Rodriguez and Bass 1985). Other studies have looked at decomposition material diffusion in soils as a method to gauge PMI (Aitkenhead-Peterson et al. 2012; Fancher et al. 2017; Carter, Yellowlees, and Tibbett 2007). From an archeological perspective, soil maps have been used to predict potential decomposition (Kibblewhite, Tóth, and Hermann 2015). Previous thesis work has looked at pH levels, microbes, and scavengers during winter decomposition in Minnesota (Bartlett 2015) and at how surface decomposition progresses during winter in Minnesota (Herbes

2023). However, most forensic decomposition studies occur in the eastern and southern United States (Emmons et al. 2022), leading to an underrepresentation of data in cooler and drier climates. Decomposition in differing soil types (clay, silt, sand, loam, organic) has been studied (Tumer et al. 2013; Parson 2019), but these studies are usually not from the perspective of soil-forming factors and take place in warmer climates (Emmons et al. 2022).

2.12 The research sites

Two sites were selected for this research project, with the intent that the main difference between the sites being land use: 1) cultivated cropland and 2) deciduous forest cover (Figure 1). The Site 1 soil was mapped as Reedslake-Le Sueur complex while the soil of Site 2 was mapped as Lester-Belview complex (Appendix 2). The sites were located in the Western Corn Belt Plains of the Des Moines Lobe ecoregion (White 2020; United States Environmental Protection Agency 2022). Within the Temperate Prairies region of the Great Plains, the Western Corn Belt Plains are known for fertile mesic soil and high agricultural use. The landscape is built upon glacial plains, loess deposits, and morainal hills. Much of the region consists of Udoll and Aquoll soils, forming wet and moist prairies. Corn, soybeans, and feed crops have largely replaced the natural tallgrass prairie, with the land being artificially drained for cultivation (Sands 2018; Minnesota Board of Water and Soil Resources n.d.).

The Des Moines Lobe ecoregion extends from southern Minnesota into central Iowa, terminating at Des Moines (White 2020; United States Environmental Protection Agency 2022; Hooyer and Iverson 2002). The Des Moines Lobe ecoregion was covered by a large

Figure 1. Aerial image of the project area. Site 1 is in the cultivated cropland and Site 2 is in the deciduous

glacier of the same name during the Wisconsin glaciation, which was the final glacial period during the Pleistocene period (White 2020; Dengler 2017). Much of this area consists of flat to gently rolling topography, remnants of the glacial retreat around 11,000 years ago (Lusardi and Dengler 2017). Glacial till deposited as the Des Moines lobe retreated. The till consists of carbonate, crystalline, and shale clast rock fragments from the northwest in nearly equal portions of clay, silt, and sand particles, forming a fine-textured loam (Lusardi, Jennings, and Harris 2011). The northwest portion of the ecoregion meets the Northern Glaciated Plains ecoregion while the northern edge transitions into the North Central Hardwoods ecoregion (Lusardi and Dengler 2017). Both sites were on the west side of the Minnesota River, at the northeast border of the Des Moines Lobe ecoregion with Heiberg and Dovray Till Member deposits, which have a loam to clay loam matrix texture (Lusardi, Jennings, and Harris 2011).

Both sites experienced an average high temperature of 28.3ºC (83°F) in July and an average low of -16.7ºC (2°F) in January (United States Climate Data 2023). The average annual precipitation in this area is 33.8in (85.85cm) with an average annual snowfall of 38.7in (98.3cm). Most of the precipitation occurs during the summer (14.21in [36.1cm]) while winter has the least precipitation (3in [7.6cm]).

Despite both sites being located on low slopes (0-3%), they were exposed to different effective precipitation, or the amount of precipitation that becomes available for soil development (Schaetzl and Anderson 2005). Site 1 was in a plowed field with no tree or grass coverage, allowing for more evaporation than Site 2, which was in a forested area. Site 1 was planted with soybeans which do offer thick ground coverage when mature, but growing to this size takes several weeks. Artificial drainage was also installed in the field where Site 1 was located, further draining the area of natural precipitation accumulation. Site 2 was on the footslope of the upland landform and is impacted by runoff from uphill, but would not pool over the site. Site 2 additionally had ground coverage from leaves which help maintain moisture, and was located on a northeast-facing slope, potentially providing more shade in the area, especially during winter months. However, the location in the valley where Site 2 was located had several hours of sun exposure during the spring and fall when the sites were created and excavated. Site 1 was heavily impacted by human use, and while Site 2 was in a natural drainage, the site was downhill from a house and was surrounded by modern discards like barbed wire fencing, plastic cups, and other trash.

Chapter 3. Methods

3.1 Basis of the experiment

This research is a quantitative experiment, looking at the weight and general appearance of ham hocks after being buried for approximately five months (5/2/23-10/8/23). Two sites within the same ecoregion, elevation, and similar soil classifications were chosen for the experiment, with the independent variable being the differing ecology acting on the soils. The samples were buried for the same amount of time, and upon excavation, the samples were compared based on their weight, appearance, and surrounding soil chemistry to evaluate the level of decomposition.

3.2 The location and setup

The research took place over the growing season of 2023 (May 2-October 8) at a site north of St. Peter, MN (Appendix 1). Six frozen ham hocks were purchased from Schmidt's Meat Market in Nicollet, MN on May 1st and were allowed to defrost in a refrigerator overnight. Ham hocks, a standard butcher cut of pork, are the joint that connects the pig's leg to its foot on the hind limbs. Ham hocks are around four inches in length and consist of bones (tibia, fibula, calcaneus, and tarsal), collagen, some muscle tissue, connective tissue, fat, and skin. On May 2nd, they were not fully defrosted, but due to time constraints, the experiment went forward as planned. Each ham hock was weighed and photographed prior to burial (Figure 2). The samples were kept in labeled gallon bags prior to burial for consistent documentation.

The ham hocks (pig samples) were buried after the snow had fully melted in the project area (though snow was observed nearby, and Barney Fry Creek had not fully melted). Three samples were buried at each site and were spaced approximately 2 m (6.5 ft) apart. Tools used for this portion of the experiment included: shovel, measuring tapes, food-grade scale, Munsell color book, compass, soil auger, and soil profile tray.

Figure 2. Ham hock pre-decomposition. Bag used for transportation and to denote sample number.

<u>3.3 Burial and retrieval of the samples</u>

Prior to burial, the soils at each site were evaluated using the auger, measuring tape, and soil profile tray (discussed more in section 3.4). Using the shovel and tarps, the ham hocks were buried in areas with a low slope (0-3%) and were spaced about 2 m (6.5 ft) apart to prevent runoff or overlap in burial fluids. The samples were buried 40 cm deep in holes with a circumference of about 30 cm. Triangulation measurements were taken to mark where each sample was buried, and flagging tape was additionally used in areas where possible.

The samples were retrieved after harvest to avoid harming the crops. The samples were excavated using the aforementioned measurements, tarps, a screen, and shovels. Excavation units were 40 cm x 40 cm in 10 cm intervals until the remains of the samples were located. The soil was additionally passed through a screen with 1 x 1cm holes if the samples were not located through digging alone. Additional soil notes were taken, specifically regarding any changes in the soil texture and color (based on a Munsell color book), and soil samples were collected from the area immediately around the pig samples. The samples were stored in a freezer bag with icepacks until the excavation was complete, then refrigerated overnight to prevent further decomposition. The next day, the samples were photographed, rinsed and lightly brushed of any residual dirt, and photographed. Each sample was re-weighed, and notes were made about any floral and faunal activity present. The Munsell color book was used to describe the colors of the soil, bones, and mold activity.

3.4 Documentation of the soils

Prior to interment of the pig samples, the soils were described. Using an auger, soil profiles were excavated to a depth of 95 cm at Site 1 and 120 cm at Site 2. (Site 1 was not excavated further due to obstruction.) The profiles were organized on a soil profile tray (Figures 3 and 4) and were described based on the Natural Resources Conservation Service and National Soil Survey guidelines (Appendices 2 and 3).

Figure 3. Site 1 soil.

Figure 4. Site 2 soil.

Soil samples were collected from both sites pre-burial and post-exhumation of the pig samples. Pre-burial, soil samples at each site were collected at a depth ranging from 0 – 40cm, were mixed into a single gallon-sized plastic bag and sealed. During excavation in October, soil samples were collected at the same depths, with attention to collecting soil in the vicinity (5 – 10 cm), and especially below the pig remains. Soil samples from pre- and post-decomposition were sent to Ward Laboratories in Kearney, NE for chemical analysis. Ward laboratories performed a "Routine Soil Analysis" on the four samples, testing pH, base saturation (%), and organic matter, electrical conductivity, nitrate-nitrogen concentration, potassium, phosphorus, magnesium, calcium, sodium, manganese, copper, iron, zinc, and sulfate content (Table 1). Previous literature has explored organic matter, nitrate, and pH levels in the context of decomposition, and because these experiments have provided a context for this research, these chemical analyses are the focus in the evaluation. However, consideration of the other chemical levels will be given as well.

One soil sample from each site was also sent to the EARTH Systems Laboratory at Minnesota State University, Mankato (MNSU) for particle size distribution analysis using a Malvern Mastersizer 3000 laser diffractometer (Table 2). Using this method, lasers are passed through the samples suspended in water and reported based on size class. One hundred and five classes, ranging from 0.01 µm to 2 mm, are observed.

Prior to analysis, the samples were dried at 105°C for at least 48 hours. The samples were then ground using a mortar and pestle and passed through a 2 mm (#10) sieve. The samples were then soaked in a 5% sodium hexametaphosphate solution for another 48 hours to disperse soil aggregates into individual particles. Once prepared, enough sediment to reach between 5 – 10% obscuration was added to the dispersion unit of the Mastersizer where the samples were sonicated for 300 seconds (5 minutes) to further disperse any remaining aggregates. Three measurements were taken with the averages calculated and used for data analysis. The results were adjusted for error caused by laser diffraction overestimating the silt fraction and underestimating the clay fraction (Bowen, 2023, personal correspondence). Based on the volume of sand, silt, and clay in the samples, soil texture was classified using a textural triangle.

3.5 Documentation of the remains

Upon excavation, the six ham hocks (three from each site) were weighed and described using the method developed by Megyesi and colleagues (2005), also known as the Megyesi method (Table 3). This method has been used to predict the PMI of decedents based on stages of decomposition but can also be used to describe the level of decomposition observed during an experiment. Megyesi and colleagues first approached decomposition by dividing the process into four categories: fresh, early decomposition, advanced decomposition, and skeletonization (2005). “1” was assigned to fresh, with the values increasing with each subsequent stage. Different parts of the body go through the stages of decomposition at different rates and were therefore scored separately before the totals were added. For the purpose of this experiment, only the scale pertaining to the limbs was applied.

The accumulated degree days (ADD) were also calculated to determine the heat available to allow biological processes to occur. ADD is calculated by taking the minimum and maximum air temperature reached in a day, adding them together, dividing them in half, then subtracting the minimum temperature threshold needed for biological activity (Figure 5; Michaud and Moreau 2011; Megyesi, Nawrocki, and Haskell 2005).

$$ADD_{thd} = \left[\frac{\{T_{\min} + T_{\max}\}}{2}\right]$$

Figure 5. ADD equation pulled from Michaud and Moreau 2011

ADD have been used in agricultural practices to determine when it is the best time for planting and harvesting crops as a certain number of ADD will need to be reached for crops to be harvested. In this instance, the minimum temperature will be the point at which plants can grow. For this experiment, Megyesi and colleagues' base temperature of 0°C was used as this is the temperature most cited when microbial activity has stopped or slowed to a point where it cannot be easily detected. Climate data for St. Peter, MN, including precipitation, was retrieved from the Minnesota Department of Natural Resources website (accessed November 16, 2023; the averages and totals are presented on Table 4). The weather readings were taken and reported from the Mankato Regional Airport (KMKT), which is the closest airport in the area. This portion of Minnesota received low levels of precipitation and was reported as experiencing a drought over the summer. Using the ADD, the degree-day index (Figure 6) was also calculated and applied to the discussion of the pig samples post-decomposition.

$$Y = -0.0124\mathrm{ADD}_{6} + 0.0112\mathrm{ADD}_{10} - 0.239\mathrm{ADD}_{12} + 0.151\mathrm{ADD}_{13}$$

Figure 6. Multiple regression equation used to calculate the Degree Day Index. Pulled from Michaud and Moreau 2011.

3.6 Methods evaluation

Previous studies have used pigs as proxies for human decomposition, and while they are no longer considered the ideal analog (Connor, Baigent, and Hansen 2018; Collins et al. 2022; Dautartas et al. 2018), they were deemed appropriate for this study for multiple reasons. The focus of the experiment was on how different soils affect decomposition. While human cadavers or even full pigs or sheep carcasses would have been preferred, this is still a preliminary study into soil forming factors, soil types, and their relation to decomposition under specific groundcovers. Due to timing, convenience, and funding, ham hocks were therefore used to compare the decomposition process. Similarly, the samples were buried deeper than ideal levels for insect and microbial activities (Campobasso, Di Vella, and Introna 2001; Emmons et al. 2022). The samples were buried at a depth that would allow for some microbial activity, but deep enough to not attract scavengers like coyotes or be disturbed by plowing. The forest site was checked multiple times in subsequent weeks and showed no signs of disturbance. The plowed field was planted with soybeans within a week of the experiment beginning and was inaccessible for observation. Initially, an additional site was a part of the experiment, but was not located upon excavation day. A GPS handheld device would have been preferable for recovery but was not available at the time of the initiation of the experiment.

Initially, the experiment was meant to be performed over the winter, but time constraints in getting permissions and setting up the study moved it to the summer. The preference for a winter experiment stemmed from the lack of research on decomposition in cold environments. However, because the study shifted focus to how soil formation impacts decomposition, the timing of the experiment was conformed to the planting and harvesting schedule.

At the time of the experiment, retrieving soil samples for chemical analysis was focused on areas with the most organic material (e.g. the Ap and A horizons, or 0-40 cm). Pre-decomposition, much of the soil sample came from closer to the surface and extended into the sub-surface environment at both sites. Post-decomposition, the soil sample was focused around and beneath the decomposed pig sample and was mixed with matrix from closer to the surface. This undoubtedly skewed the results and was an oversight at the time. Future studies should take not only multiple samples from each site but take samples at consistent depths. Additionally, soil samples from the sites and out of range of the decomposing material should be taken post-decomposition as a control to observe the seasonal chemical changes in the soil.

Chapter 4: Results

4.1 Megyesi evaluation, Accumulated Degree Days (ADD) and Precipitation

Using the Megyesi method of evaluation, the remains were determined to be completely, or nearly completely skeletonized (Figure 5). While adipocere had formed on the ham hocks, the bones from all the samples at both sites were exposed upon excavation.

The buried pig samples were exposed to a total of 3,262 accumulated degree days (Table 4). The degree-day index was calculated at 21.87, well above the index value predicted by Michaud and Moreau (2011) for the onset of dry decay.

Sample	Pre-Experiment Description	Post-Experiment Description	Score
1.1	Fresh, no discoloration. Flesh and connective tissue surrounding bone. Fat layer between flesh and skin.	Bone completely exposed. Some adipocere formation around cavity where pig part was located. Bone still greasy, not dry.	9
1.2	Fresh, no discoloration. Flesh and connective tissue surrounding bone. Fat layer between flesh and skin.	Bone completely exposed. Some adipocere formation around cavity where pig part was located. Bone still greasy, not dry.	9
1.3	Fresh, no discoloration. Flesh and connective tissue surrounding bone. Fat layer between flesh and skin.	Bone completely exposed. Some adipocere formation around cavity where pig part was located. Bone still greasy, not dry.	9
2.1	Fresh, no discoloration. Flesh and connective tissue surrounding bone. Fat layer between flesh and skin.	Much more adipocere formation, completely encasing bone.	8
2.2	Fresh, no discoloration. Flesh and connective tissue surrounding bone. Fat layer between flesh and skin.	Bone exposed more than other forest area sample, but more adipocere present than Site 2 examples.	8
2.3	Fresh, no discoloration. Flesh and connective tissue surrounding bone. Fat layer between flesh and skin.	Bone exposed more than other forest area sample, but more adipocere present than Field examples. Difficult to score because it was not found intact.	8

Figure 7. Megyesi categorization applied to the pig samples.

4.2 Descriptions of the remains pre-decomposition

The six ham hock samples acquired for this experiment started out around the same size and weight, ranging from 354 to 474 grams (average of 412g; Appendix 4). All the samples had light pink skin over a fat layer. The fat layer was separated from the bones by muscle and connective tissue. Bone marrow was visible where the samples were cut, and some samples still had hair attached to the skin. Very little blood was present.

<u>4.3 Descriptions of the remains post-decomposition</u>

The three ham hock samples in the plowed field (Site 1) showed signs of adipocere formation (Figure 8; Table 5). White invertebrates, likely springtails (*Coecobrya tenebricosa, Entomobrya confuse, or Fulsomia Candida* [springtails.us. n.d.]) and bulb or soil mites (of the Family Acaridae), possibly pot worms (of the Family Enchytraeidae), and tiny brown spiders (of the Order Araneae) were present in varying amounts, with the first sample having the most activity (Appendix 5). The soil directly around the bones had a greasy texture. Bones were darkened in certain areas, but especially on the

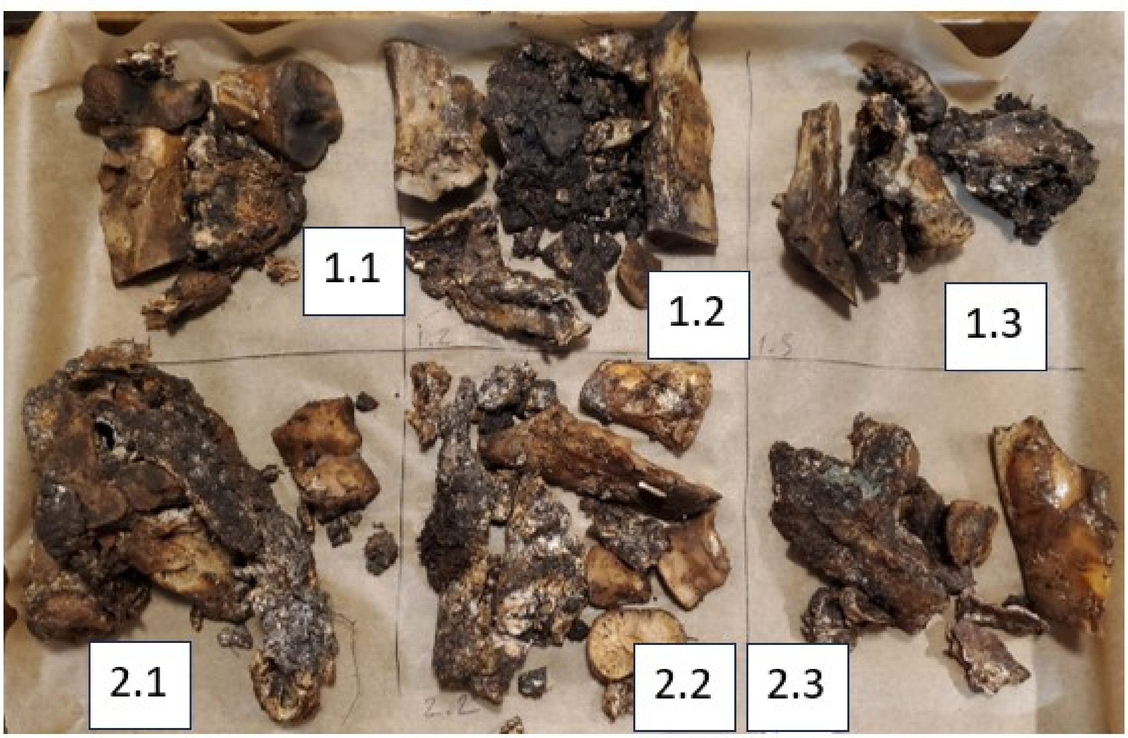

Figure 8. The decomposed pig samples after cleaning. Numbers indicate Site and sample number from the Site.

proximal ends. Using the Munsell color charts, the colors of the bones were found to range from white (7.7YR 8/1) to black (5YR 2.5/1), with dark gray (10YR 4/1), and dark brown (10YR 3/3) and other similar shades varying in between. The adipose tissue was very white (N9.5).

The first sample from Site 1 (sample 1.1) had the most invertebrate (mites and springtails are not true insects) and spider activity (Figure 6). The springtails were found in abundance all over the remains during excavation, and after a day in refrigeration additional mites were present. At the cut end of the calcaneus, a yellow (10YR 8/8) fungus was observed. Some dry flesh and hair were present on the anterior side of the tarsal. The tiny brown spiders and springtails were also present on the second sample from Site 1 (sample 1.2), but in fewer numbers (Appendix 7). A large piece of adipocere (approximately 10 cm in diameter) had formed around the bone and was difficult to separate fully from the soil. Some soil had fused to parts of the bone that felt greasy, as if the fat had melded with the soil. More fungus was growing directly on the bone in this sample as well. While most of the other bone fragments from both sites did not show any additional decay and generally looked fresh, if stained, the anterior portion of the calcaneus from this sample showed decay on the cortical bone with exposed trabecular bone (Appendix 9). The final sample from Site 1 (1.3) had the least amount of invertebrate activity and no evidence of springtails or mites (Appendix 8). White and yellow fungi were present and less adipocere tissue had formed. After initial cleaning and observations, the remains were placed in a cool garage for storage. After two days, invertebrates were still in the remaining sediment and around the pig remains but had not spread to the samples from Site 2, even though no barriers were in place.

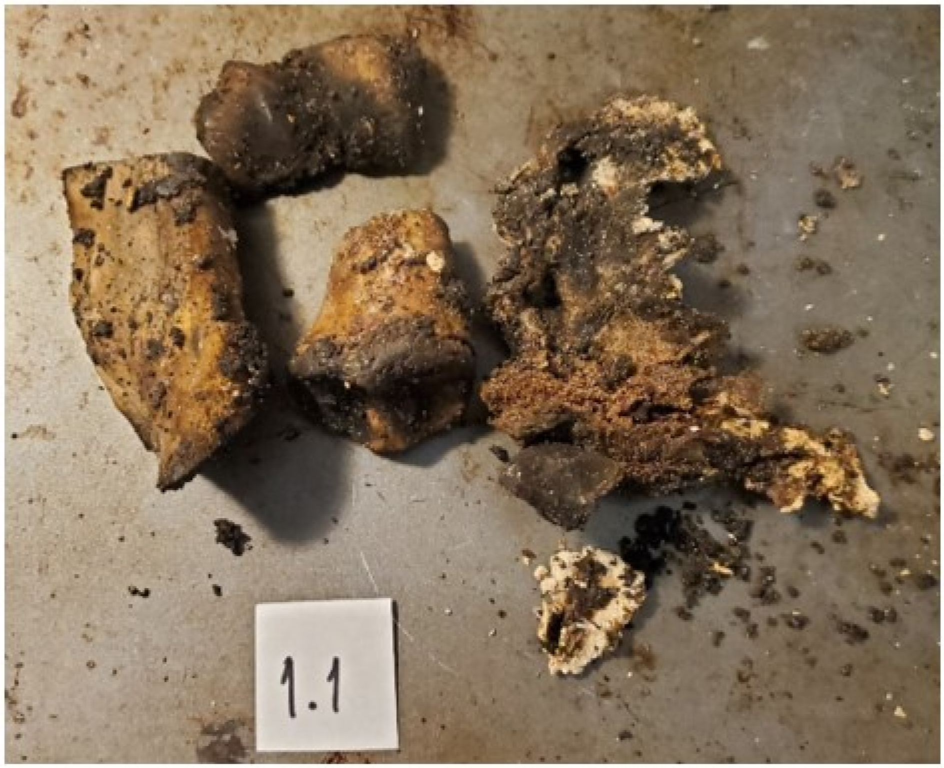

Figure 9. Pig sample 1.1. Mites abundant on adipocere tissue.

All samples from Site 2 had signs of adipocere formation, but little insect or other invertebrate activity. Some larvae, probably belonging to the order Coleoptera (beetles), were observed. Roots were heavily present surrounding the samples and the soil directly around the bones had a greasy texture. During excavation, adipocere encased root growth, sediment, and bones in situ, forming the shape of the original ham hocks (Figure 10). Site 2 samples were stained dark brown (10YR 3/3) and black (5YR 2.5/1) in part, but especially on the proximal ends. Dark reddish brown (2.5YR 3/4) adipocere tissue was also observed in areas where the tissue was directly touching the bone. Bones covered in adipocere tissue also appeared to be less decayed than bare bone. Drier adipose further from the bone was more of a pale brown (2.5Y 8/4) color.

Figure 10. Pig sample 2.1 during excavation. Adipocere tissue encased roots, soil, and bones, forming the structure of the original ham hock.

The first sample from Site 2 (2.1) had little to no invertebrate activity, but some small larvae were observed. Much adipocere tissue was present on this sample (around 20cm in length and 10cm in width), and when in situ, soil and roots filled the area between the adipocere and the bones (Figure 10). This sample was collected with more soil intact initially to try to preserve the original shape. Adipocere was in chunks of the surrounding soil in addition to encasing the sample and were difficult to separate from the bone without discarding potential tissue. Fungus was also present on the bones (Figure 11). The soil was greasy and dark brown (10YR 3/3). Once cleaned, hairs from the pig skin were observed still present on distal ends of the calcaneus. The second sample from Site 2 (2.2) was very

Figure 11. Pig sample 2.1. Adipocere tissue was well-formed and fungus was present on the bones.

similar to the previous sample with soil and roots attached to the bones, white adipocere tissue surrounding the bones, and little to no invertebrate activity (Appendix 9).

The final sample (2.3) was partially damaged during excavation and was missing the metatarsals (Appendix 10). The tarsus was slippery (Figure 8), and a dark greenish gray (5GY 3/2) mold was observed on the surrounding dark, greasy soil that was fused with the adipocere tissue. The distal end of the calcaneus was chipped during excavation, revealing a similar color (grayish brown) to other bones on the cortical bone and white trabecular bone, but a thin layer between the two was stained dark brown (7.5YR 3/3; Figure 12).

No fresh or decayed flesh remained on any of the samples post-decomposition. Some dry, possibly mummified flesh or skin with hair was observed and adipocere tissue had formed, but muscle and connective tissues were absent. Additionally, the adipocere formation on most of the remains was not attached to the bone but had encased the area

Figure 12. Chipped calcaneus from pig sample 2.3. The exterior bone is grayish-white like the other bones and the inner spongy bone is white, but a thin layer between the exterior and interior is stained dark brown.

surrounding the bone. The adipocere, especially in the Site 2 samples, appeared to form in the areas where the fattiest tissue had previously been on the original ham hocks, directly beneath the skin and separated from the bones by muscle and connective tissue.

The samples from Site 1 lost an average of 307g, weighing about 25% of the original weight. Site 2 samples lost on average 281g, with a weight approximately 31% of the original weight after decomposition. Both sites lost an average of 294g, or 28% of the total weight. It should be noted again that the final sample of Site 2 was partially damaged during excavation, so more weight is possible for this set. Additionally, it was impossible to completely remove the soil from the samples without further disintegrating the remaining adipocere, likely increasing the weight of the samples. Despite the adipocere formation, all of the samples would still qualify as being totally skeletonized by the Megyesi method as no flesh was directly attached to the bone.

<u>4.5 Soil descriptions from the field analysis</u>

No major changes in the soils were noted during exhumation. Site 1 soil (Appendix 2) was very moist during the initiation of the site due to snowmelt and was somewhat moist during excavation from recent precipitation. The site consisted of an Ap horizon over an A horizon and two Bt horizons with abrupt boundaries between the Ap, A, and the first Bt horizon (Figure 3). Site 2 (Appendix 3) consisted of A-AB-Bt-Bt2 horizonization with gradual boundaries (Figure 4). Site 2 was downhill from a house and modern discards like barbed wire fencing, plastic cups, and other trash were present.

Site 1 had a relatively thick Ap horizon, extending to 26 cm. The soil was black (10YR 2/1) with a firm consistence, and a strong, coarse, subangular blocky structure. There was evidence of previous corn crops but little to no additional rocks or roots. The soil had a silty clay hand texture and an abrupt boundary above A horizon. The A horizon was very similar to the Ap horizon, with a slight color change to very dark gray (10YR 3/1) and an increase in clay content and mottles. At 47 cm, the soil transitioned into a Bt1 horizon with mottles of very dark grayish brown (10YR3/2) and grayish brown (10YR 5/2) colors. The structure and consistence were similar to the previous horizon, but the clay content increased, forming a clay loam. Additionally, some small rocks were found in this horizon. The Bt2 horizon began at 71 cm, with more mottling and redoximorphic features. The soil was yellowish brown (10YR 5/4) with strong brown (7.5YR 5/8) dispersed throughout. The structure grade and type remained consistent with the previous horizons (strong subangular blocky), but the size increased to very coarse. More rocks were found in this horizon and the profile description was ultimately terminated due to obstruction by glacial till at 95 cm.

The forested area (Site 2) had a very dark gray (10YR 3/1) A horizon that went down to 34 cm. The structure of this horizon consisted of moderate, medium subangular blocky soil units, with fine roots throughout. The soil was a friable silty clay, with a gradual boundary that transitioned into an AB horizon. The AB horizon was similar to the topsoil, with the main difference being an increase in clay, but still having a silty clay loam hand texture. A Bt horizon (Bt1) was observed at 74 cm, with a shift in color to very dark grayish brown (10YR 3/2) and an increase in roots. This horizon extended to 104 cm before transitioning into a second Bt horizon (Bt2). The Bt2 horizon had the same color, structure, and consistence of the Bt1 horizon, but had more of a silty clay hand texture, similar to the first two horizons. Additionally, roots increased in size and some rocks and gravels were observed. The profile was terminated at 120 cm, which was the length of the auger being used.

4.6 Particle analysis results

Both soil samples were classified as silty clays based on particle size distribution data. The clay content in Site 1 was slightly higher than in Site 2 (47.77% in the plowed field versus 41.1% in the forest; Table 1). The soil from Site 2 area had a slightly higher sand content (8.89% in the forested soil versus 4.01% in the plowed field). The percentages for silt were much closer, with a difference of only 1.8%.

4.7 Ward Laboratories results

The chemical analysis performed by Ward Laboratories revealed a decrease in the active soil pH and the total soil pH levels between the start of the experiment and the excavation at Site 1 (6.7 to 5.7 and 7.2 to 6.8, respectively; Table 2) with a larger difference observed in the active soil pH. The soil pH at Site 2 increased in both pH measurements (6.2 to 6.7 and 6.7 to 7.2, respectively). Similarly, the soluble salts, nitrate levels, and percent saturation of hydrogen increased at Site 1 but decreased at Site 2. Both sites showed decreases in organic matter (OM), iron, and magnesium content. Potassium content, decreased at Site 1, but remained the same at Site 2, while the percent saturation of potassium, calcium, and magnesium decreased at Site 1 and increased at Site 2. The percent saturation of hydrogen increased at Site 1 and decreased at Site 2. Both soils showed an increase in the ammonium acetate extractable cations of magnesium and the percent base saturation of sodium. The electrical conductivity increased from low to medium levels at Site 1, but decreased from medium to low levels at Site 2 (1:1 Soluble Salts mmho/cm on Table 2).

Using the organic matter results from Ward Laboratories, the dissolved organic carbon (DOC) percentage of the soil can also be estimated. This is done by taking the OM% and multiplying it by 0.58. Site 1 was estimated to be 3.19% and 2.9% DOC pre- and post-decomposition, respectively. Site 2 was estimated to be 4.06% and 3.54% DOC pre- and post-decomposition, respectively. Unfortunately, without knowing the percentage of dissolved organic nitrogen (DON) in the soil, the C:N (carbon to nitrogen) ratio in the soil was not calculated.

4.8 Agricultural chemicals used on Site 1 pre- and peri-experiment

The herbicide Authority First was sprayed on the plowed field at the time of planting (Brandon Hager (farmer), personal correspondence. November 2023). The active ingredients in Authority First are sulfentrazone and Cloransulam-methyl, both designed to suppress and control weeds and grasses. The herbicides Glyphosate, Enlist, and Dual were applied in a single application in mid-June. The active ingredients in these herbicides are *N*-(phosphonomethyl) glycine, 2,4-D, and S-metolachlor, respectively. Nitrogen in the form of anhydrous ammonia was applied to the soil in the fall of 2021 and is usually applied every other year. The field is not irrigated and has some drainage tile installed to prevent natural water logging.

Chapter 5: Discussion

5.1 Initial findings

Based on the visual results from the excavation, adipocere tissue is more likely to form on remains in forest soils. The samples all had similar levels of skeletonization, but more adipocere tissue was present on the samples buried at Site 2, the forested area. More invertebrate activity was observed on the samples from Site 1. While there were some insects, especially larvae, on the Site 2 samples, the first sample from Site 1 (1.1) was inundated with springtails (possibly *Coecobrya tenebricosa, Entomobrya confuse, or Fulsomia Candida*), and bulb or soil mites (of the Family *Acaridae*). Thin white worms, possibly pot worms (of the family *Enchytraeidae*), and tiny brown spiders (of the Order Araneae) were also present. Site 2 samples showed much more floral activity with roots, likely from the surrounding trees, filling the space between the adipocere tissue and the bone. The first sample collected from this series (2.1) had the most root activity but was also closest to a large hardwood tree, and the soil directly around the samples had a greasy texture when compared to soil further away and the soil pre-decomposition.

5.2 Decomposition related to location and moisture

More adipocere formation on the forest samples than the samples in the plowed field was predictable due to the increased protection from the sun. The samples were on a north-facing slope in the northern hemisphere, and were therefore exposed to less sunlight than the samples in the field (Schaetzl and Anderson 2005). Less sun exposure prevents evaporation, increasing moisture content in the soil. Additionally, while the tree coverage may have prevented some precipitation from directly impacting the forest soil, the slope would have allowed for runoff from uphill to reach the sample area and inundate the soil.

The soil at both sites was determined to be a silty clay, creating an environment where water is more easily trapped than if the soils had been coarse-grained (Schaetzl and Anderson 2005; Al-Kaisi et al. 2017). The soils were mapped as soils with moderate permeability (USDA Natural Resources Conservation Service, n.d.), which is consistent with glacial till as it can have low permeability (Clarke 2018). Based on the particle size data, the soils at both sites appear to hold more closely to the traits of glacial till with the fine-grained soil inhibiting movement of water that created an anaerobic environment, ideal for adipocere formation (Tsokos and Byard 2016). The temperature of the soil was also likely impacted by the moisture content. The electrical conductivity was low to medium at both sites (*1:1 Soluble Salts mmho/cm* on Table 2), likely preventing major fluctuations (Schaetzl and Anderson 2005).

The accumulated degree days for the area during the experiment time were calculated at 3,262 ADD with a degree-day index of 21.87 which is well above the threshold for the onset of dry decay (Michaud and Moreau 2011). Because the samples were buried at 30-40 cm, daily temperature fluctuations were unlikely (Schaetzl and Anderson 2005). The soil temperature was also likely to be at least 2°C off from the air temperature, though depending on the season, this could be 2°C higher or lower. While it is likely that the soil at this depth was cooler than the air temperature, especially during the height of the summer, it is unlikely that the differences in temperature had a greater effect on the decomposition process than had the temperature been the same as the air temperature. All the pig samples had reached similar levels of skeletonization, which is consistent with temperatures that allow full decomposition.

Within the literature, continuous exposure to moisture is the most cited cause of adipocere formation (Emmons et al. 2022; Byers 2017; Perper 2006; O'Brien and Kuehner 2007; Tsokos and Byard 2016). Bacteria is also important to adipocere formation (Tsokos and Byard 2016), which in turn is affected by agricultural practices (Upchurch et al 2008; Wu et al. 2023) and typically thrives in moist, warm environments. While crop soils can have more diverse bacteria populations, developed forest soils have been found to have more abundant bacterial activity, therefore potentially promoting adipocere formation.

5.3 Decomposition and the chemical analysis of the soils

While the literature supports adipocere formation in forests based on the abundance of bacteria, adipocere is also more likely to form in mildly alkaline soils (Ubelaker 2023). This was not observed in the present study, as the active soil pH and the total soil pH at both sites remained within neutral ranges (pH 5.5-7.5; Table 2). The active soil pH of Site 1 decreased by a full degree, becoming more acidic post-decomposition. However, the total soil pH value only decreased by 0.4. The total soil pH is also the value needed for determining when to apply lime to fields (Barrera 2017). Previous studies comparing forest soils to crop soils have indicated that the pH in organic soils can decrease after decomposition of mammal remains (Tumer et al 2013; Haslam and Tibbett 2009) though other studies have shown soil pH to increase when exposed to a decomposing cadaver (Tibbett and Carter 2009; Haslam and Tibbett 2009). No correlation between pH influxes and decomposition has also been observed (Fancher et al. 2017). All the pH readings remained within a neutral range (5.5-7.5) as well, so while there were differences, they are not levels that have been considered acidic or alkaline in past research (Haslam and Tibbett 2009) or by farmers testing their soil (Hammac 2021). Without further, more frequent, testing of the project areas, it is difficult to determine the true cause of the changes in pH for this experiment.

Nitrogen additions from inorganic fertilizers (nitrate) and soybean crops are also known to decrease turnover rates of soil organic matter (SOM; Mahal et al. 2019; Bonner et al. 2019), potentially preventing decomposition. This delay may have been observed in a shorter study period, but the experiment window was wide enough to allow both sets of samples to reach skeletonization. The nitrate in the Site 1 soil was shown to be lower than the forest soil at the start of the experiment but increased considerably during the experiment while the forest soil was shown to decrease during the experiment. The increase in nitrate at Site 1 could be attributed to artificial additions from fertilizer (Hall, Russell, and Moore 2019), but anhydrous ammonia (nitrogen fertilizer) was added over a year before the experiment began (Brandon Hager, personal correspondence, November 2023). While this could be a contributing factor and a sampling error could have collected more nitrogen during the post-season sample, the season's crop was soybeans. Inorganic additions of nitrogen from soybeans at Site 1 are more likely (Peralta and Wander 2008).

Both sites saw a decrease in soil organic matter content with Site 1 only decreasing by 0.5% while Site 2 decreased by 0.9%. This could be related to the soybean crop planted over the summer as this has been shown to increase decomposition when rotated with corn coverage (Hall, Russell, and Moore 2019). Dissolved organic nitrogen, which also increases decomposition (Peralta and Wander 2008), has been associated with electrical conductivity (Aitkenhead-Peterson 2012). Because the SOM decreased at Site 1 where there was an increase in electrical conductivity, this could indicate that more dissolved organic nitrogen was present, increasing decomposition, and thus reducing SOM. However, there was a greater decrease in SOM at Site 2 where the electrical conductivity also decreased and more adipocere tissue was present, both of which contradict previous expectations regarding SOM.

One explanation for the discrepancies is that SOM is used by plants as a source for nitrogen (Shober and Taylor 2014), meaning the surrounding vegetation could have sought out the SOM and therefore decreased the amount left in the soil. Additionally, soybeans are known to absorb available nitrogen from the soil before ‘fixing’ the nitrogen in the air (Killpack and Buchholz 2022). If nitrogen was available in the soil when the samples were deposited, the soybean crop likely did not absorb as much of the organic matter as a source for nitrogen when compared to the trees at Site 2. However, these discrepancies also likely indicate sampling errors as only two soil samples from each site (one pre- and one post-decomposition) were analyzed. The initial soil samples included soil from near the surface extending into the subsurface horizons (0-40 cm). The second round of samples included soil from closer to the surface and soil from near the pig samples (5 – 10 cm radius), and therefore potentially further from the surface than the initial soil samples. While more organic matter could have been present from the pig samples, more organic matter is naturally available in soils at surface level (Schaetzl and Anderson 2005). Spring is also when higher levels of organic matter in soils are observed as organic matter that has been frozen is able to decompose with snow melting.

Because the organic matter had decreased at both sites, the dissolved organic carbon (DOC) was also estimated to decrease proportionally. However, dissolved organic nitrogen (DON) was not measured during this study, so the C:N (carbon to nitrogen ratio) was not calculated for this project area, though based on the previous research, the ratio can be estimated to have widened over the course of the study. Previous studies have shown that the C:N is narrow in both human and pig cadaver CDIs, when fresh (Carter and Yellowlees 2007), and widen during extended cadaver decomposition, possibly as a result of an increase in carbon content and nitrogen mineralization in response to the decay (Carter and Tibbett 2006). In the context of pig farming and composting, sawdust has been advised as a carbon addition to reach the ideal C:N (25:1) for total decomposition, but an exact C:N ratio is not necessary for total decomposition of pig remains (Lim and Zulovich 2018). For composting, quick decomposition is also desirable, so artificially adjusting the C:N ratio is likely to be encouraged. Without DON and DOC measurements for this experiment, it is impossible to say exactly what the C:N was, but based on the previous research, it was likely lower than the ideal for total decomposition as bone and adipocere tissue from the pig samples were still present at both sites.

Additionally, phosphorus, calcium, and magnesium have been shown to increase in previous cadaver studies (Emmons et al. 2022; Aitkenhead-Peterson et al. 2012) and elevated phosphorous has been associated with adipocere formation (Carter, Yellowlees, and Tibbett 2007). During this experiment, calcium only increased at Site 2 and magnesium did not increase at all. The phosphorus levels, however, did increase at both sites, and could therefore be related. Manganese, which is associated with cadaveric breakdown (Carter, Yellowlees, and Tibbett 2007), did increase at both sites, and potentially stained the bones at both locations (Dupras and Schultz 2013), though tannins from organic materials in the soils are also a possible culprit (Pollock, Pokines, and Bethard 2018).

5.4 Pedoturbation and decomposition

The pig samples were buried between 30 and 40cm at both sites, well within the A horizons of the soils and therefore, in areas with high organic matter. Site 1 had a thick Ap over A horizonization, indicative of a thick natural topsoil and long-term plowing (Hartemink et al. 2020). A Bt horizon with an increase in clay content immediately followed the A horizon, potentially slowing moisture percolation deeper into the soil (Schaetzl and Anderson 2005; Schoeneberger et al. 2011). Evaporation at Site 1 was likely encouraged, however, as the size of the soybeans, especially before reaching maturity, would not provide the same shade coverage as the trees and shrubs at Site 2.

Site 2 had a thick A horizon (0–34cm) over an even thicker AB horizon (34–74cm) indicating organic processes were occurring well past the burial depth of the pig samples. The gradual boundaries of the horizons also indicate that the soil formation has not been recently reset, was stable enough to form an organic horizon, and had little to no anthropogenic, colluvial, or alluvial forces interrupting its formation. The lack of disturbance likely allowed for the forest growth, whose roots overtook the pig samples once in the ground. The root growth was expected as previous studies have shown that decaying organisms provide a ‘pulse of nutrients’ to the surrounding environment and encourage vegetation growth (Carter, Yellowlees, and Tibbett 2007). The root systems of the forest also help stabilize the soil while the vegetation above-ground prevents runoff from stripping away organic deposits like leaves and animal fecal matter. This in turn traps the organic materials which are eventually added into the soil.

5.5 Particle size and decomposition

Particle size analysis revealed both soils to be silty clays, which is consistent with the descriptions of Reedslake-Le Sueur complex and Lester-Belview complex soils (Soil Survey Staff 1998, 2006a, 2006b, and 2008). The texture of the soil was also consistent with glacially deposited parent material, which tends to have high silt and clay content (Lusardi, Jennings, and Harris 2011)

Fine-textured and clayey soils have been shown to prevent cadaver breakdown and be more conducive to adipocere formation (Carter, Yellowlees, and Tibbett 2007; Tumer et al. 2013). The smaller particle size has a lower rate of permeability, thus holding in more moisture than sandy soils and preventing oxygen – carbon dioxide exchange. Anaerobic microorganisms, which are less efficient at decomposition, thrive in these soils and further slowdown the rate of decomposition. Additionally, clay has a larger surface area when compared to the other soil textures and is more likely to bind to organic matter (Schaetzl and Anderson 2005). Both organic matter and clay are also more likely to hold in moisture (Schaetzle and Anderson 2005), which encourages adipocere formation (Wescott 2018). The high clay content and adipocere formation at both sites was consistent with this phenomenon.

5.6 Floral and faunal activity and decomposition

White mold (likely of a saprophytic variety [FoodCycler Blog, n.d.]) growth was also observed on the bones, though there were no major differences in the placement and color between the sites, other than bright yellow mold (likely a plasmodial slime mold [National Park Service 2020]) which grew in the proximal end of the sample 1.1 calcaneus in the spongy bone. Similar mold was found on other bones, but it was most prominent in this sample. The pH was not less than 5, so it is unlikely that the mold present was a major contributor to the decomposition process at either site, though the growth may have contributed to the greasy humus surrounding the samples (Schaetzl and Anderson 2005; Byers 2017), as mold belongs to the kingdom Fungi. Green mold growth was observed on the adipocere-fused soil in Site 2 (pig sample 2.3), being the only sample with this color of mold observed and mold observed not on the bone. Mold growth is more common in damp environments, further indicating the area maintained more moisture than Site 1, at least initially, though similar evidence from the surrounding samples would be needed for more definitive conclusions. The neutral pH of both sites does indicate that bacteria were the main contributors to decomposition (Schaetzl and Anderson 2005; Byers 2017; Haslam and Tibbett 2009).

There was an abundance of invertebrate activity on the Site 1 samples when compared to Site 2. While some larvae, possibly from a beetle (Coleoptera) species, were present on Site 2 samples, the Site 1 samples, especially Sites 1.1 and 1.2, were covered in invertebrates (Figure 8). A likely identification for the invertebrates observed were springtails (possibly *Coecobrya tenebricosa, Entomobrya confusa, or Folsomia Candida*), and bulb or soil mites (of the Family *Acaridae*). Thin white worms, possibly pot worms (of the family *Enchytraeidae*), and tiny brown spiders (of the Order Araneae) were also observed but were not at the same level of abundance as the springtails and mites. Springtails and mites have been observed on interred remains (Merritt et al. 2007) and beetle species are commonly cited as a main contributor to decomposition (Byers 2017; Emmons et al. 2022). Springtails are detrivores, meaning they feed on dead organic material (Missouri Department of Conservation. n.d.; New England Herpetoculture. n.d.). Springtails prefer decomposing plants, but fungus, bacteria, and decomposing animals are also food sources for some species. Additionally, mites can be attracted to food sources like dog food, mushrooms, and fish flakes (i.e. non-plant material) and can even out-compete springtails in the terrarium setting. This indicates that at least one of the species present was attracted to the pig organic material. The combination of springtails and mites at Site 1 could also indicate that both species were consuming the adipocere tissue (and possibly the fresh flesh prior) and therefore decreased the amount of adipocere initially left in the soil.

One explanation for the abundance of springtails on the pig samples at Site 1 as opposed to Site 2 could be the organic matter content in the respective soils, with Site 2 having more organic matter both pre- and post-decomposition. This could encourage scavenging invertebrates to seek out the abundant food source at Site 1 and not Site 2, as other organic food sources were available and more spread out at Site 2. Another explanation could be related to herbicides and fertilizers used on Site 1, which could change the presence of invertebrates at the site. However, the herbicides used are designed to target wide-leaf plants, not invertebrates and nitrogen fertilizers are designed to encourage plant growth. Further investigations could look at potential changes in invertebrate activities based on these additions.

The root growth in the cavities of the Site 2 samples was expected as decomposing organic matter creates a concentrated "island of fertility" (Carter, Yellowlees, and Tibbett 2007). Plants responding to the nutrient burst is consistent with previous research on the subject. Similarly, the samples were buried within the A (organically active) horizons of the soils, which would allow for the most bioactivity when compared to deeper horizons (Schaetzl and Anderson 2005).

5.7 Conclusions and recommendations

Overall, the results from this study were consistent with previous findings, especially given the timeframe, soil type, and season the remains were buried in. The soil was shown to be healthy overall in both locations, promoting microbial and invertebrate decomposition during the summer. While moisture entrapment likely promoted adipocere formation and slowed decomposition in the forest soil, the non-fatty portions of the ham hocks were completely desiccated. The results therefore support existing theories about decomposition.

The results are useful for increasing the understanding of the relationship between soils and decomposition. The soil type promoted the formation of adipocere tissue on both samples with the samples located in the forest (Site 2) showing more tissue formation. This is consistent with forested areas preventing evaporation and therefore allowing for a more steadily moist area when compared to crop soils. Additionally, factors like time, climate, and floral and faunal activity appeared to have more of an impact on the remains than the chemicals added to the crop soils because the samples were not observed immediately after chemical treatment.

The soil analysis was useful for review, there were no clear patterns between the chemical analysis and previous studies, indicating more research into chemical analysis, comparing crop soils to undisturbed soils would be beneficial. While previous studies have observed the movement of gravesoil chemicals in relation to the remains (Emmons et al. 2022) and have therefore incrementally taken soil samples for chemical testing, in this study, post-decomposition soil samples were taken relatively close to the remains (within 5-10 cm) to better capture soil affected by the decomposing pig samples. As mentioned, this likely formed an unintentional sampling bias, which favored organic matter and related chemicals found in the soil in May. Sampling at both surface level and burial depth, pre- and post-decomposition could account for these errors in future experiments. Additional control sampling from the same areas being tested, but far enough away to be influenced by the decomposing materials could be taken post-decomposition as well. This would provide a better representation of the chemical makeup of the soils and further explain the processes that occurred during decomposition.

Future research could include more segmented observations, excavating in increments of weeks or days rather than several months. This would be useful in establishing better PMI estimates in different soils and establishing a firmer timeline on adipocere formation in soil as it dries. Soil temperature could also be taken to better show how soil temperature differs from the air temperature ADD and how that might further affect decomposition. While the Megyesi method is useful in determining PMI for remains with flesh, remains that have been completely skeletonized are not covered by the method (Megyesi, Nawrocki, and Haskell 2005). Creating a similar method to predict the PMI based on skeletal remains could potentially be a future area of study. The causation of bone staining could similarly be explored. Manganese and organic matter were both found in the sample soils and both are credited with causing bone staining (Stathopoulou et al. 2019; Dupras and Schultz 2013; Pollock, Pokines, and Bethard 2018). Future tests could measure the levels of manganese and tannins in the soils and create a timeframe for staining to appear after exposure. Other areas that could be improved include having a larger sample size, testing other soil types, and using GPS and monitors to geo-locate the areas. This experiment also relied on soil tests designed for testing crop soils based on traditional American farming practices, which tested inorganic nitrate content of the soil, but did not test carbon or nitrogen content, or how much of the organic matter in the soil was occupied by bacteria, fungi, and other microbes.

Summary

This experiment revealed that mammal remains (ham hocks) can decay to skeletonization during the summer months in Minnesota in both plowed and forest soils. While adipocere tissue is more likely to form in forest soils than in agricultural soils, the processes that affect the soils (climate, organisms, relief, parent material, and time) allow for, and even promote, decomposition. The silty clay soil in south-central Minnesota additionally insulated the soil, providing a moist, anaerobic environment for adipocere formation. Future studies could shorten the experiment to establish increments for decomposition and adipocere formation based on soil formation. More research that combines soil forming factors, especially in temperate climates is needed to enhance decomposition research.

Citations

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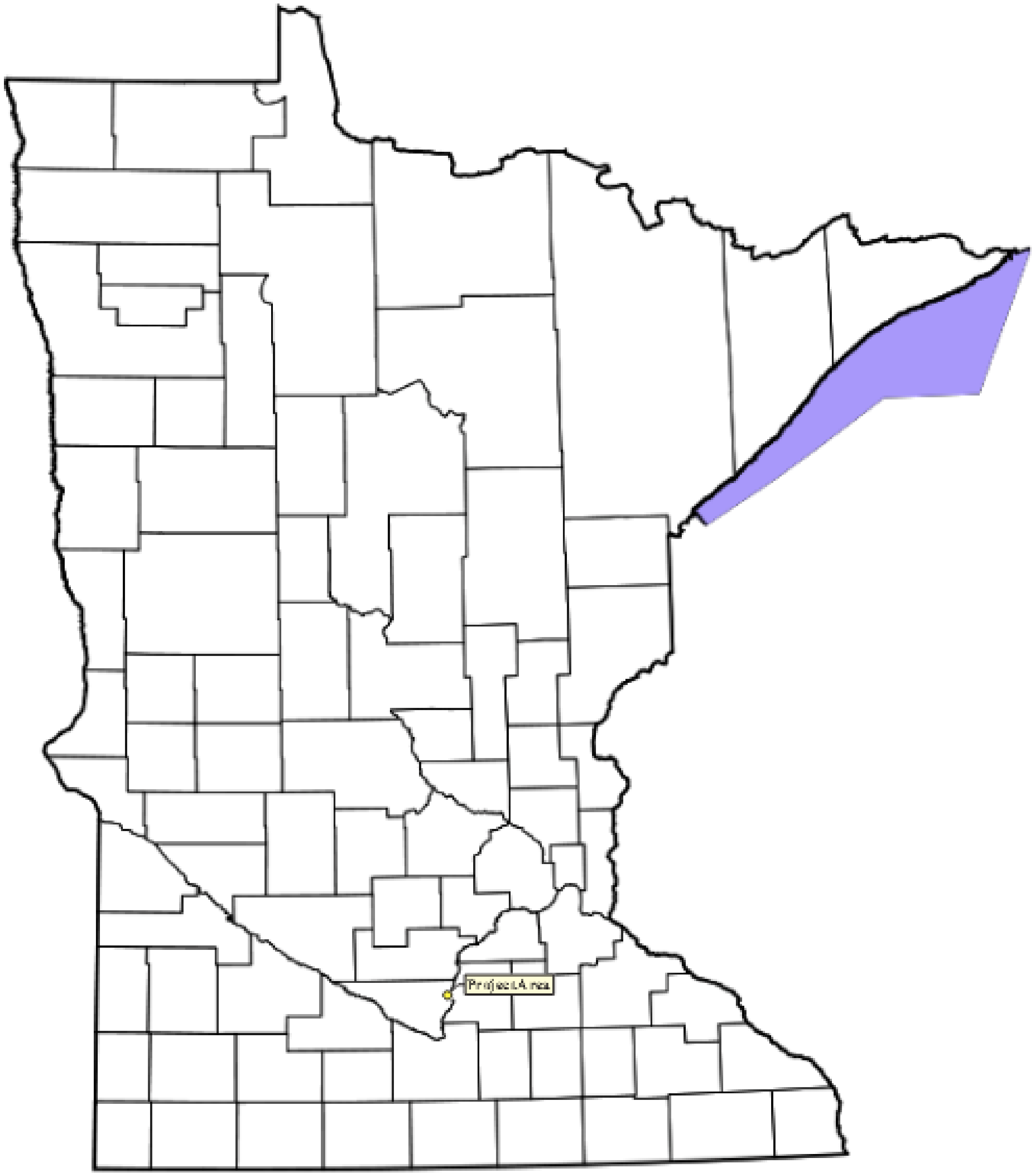
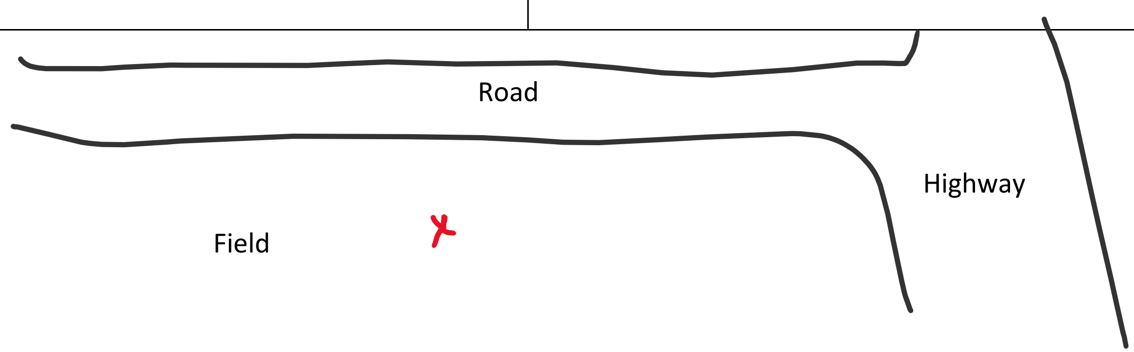
Appendix 1: Location of the Sites



Appendix 2: Geomorphic Site Description for Site 1

Site ID: Site 1, Plowed Field	**Date:** 5/2/2023	**Describers:** Haley Madden	
Current Weather: Sunny, windy, 52°F (11.1°C)	**Past Weather (48 – 72 hours):** High 57-58°F (13.8-14.4), Windy, no precipitation		
Series / Map Unit Name & Symbol: Reedslake-Le Sueur; L113B; 1-6% slope			
Taxonomic Classification: Fine-loamy, mixed, superactive, mesic Typic Argiudolls			
County & State: Nicollet County, MN	**Latitude & Longitude (DD or UTM):** 44.407172, 94.006194		
Land Resource Region: Cropland, pasture, woodland	**Major Land Resource Area:** 103 – Central and South central MN		
Avg Annual Temp (°F & °C): 7.2-10C; 45-50F	**Avg Annual Rain (in & cm):** 32in; 81cm	**Avg Annual Snow (in & cm):** 43in; 109cm	
Min Avg Temp and Month (°F & °C): -12°C; 8.8°F; January	**Max Avg Temp and Month (°F & °C):** 35°C; 95°F; June		
Soil Temperature Regime: Mesic (8C < MAST < 15C)	**Soil Moisture Regime:** Udic		
Soil Moisture: Moist	**Effective Precipitation:** Slightly less; high evaporation rate due to lack of tree coverage		
Geomorphic Environment: Slope/ Glacial	**Landscape:** Upland/ Till plain	**Landform:** Plain/ ground moraine	
Microfeatures: None	**Anthro Features:** Plowed field with corn remnants about 100 ft from paved road		
Slope Aspect (°): 85°	**Slope Gradient (%):** 0-1%	**Slope Complexity:** Simple	**Slope Shape:** Linear convex
Elevation (ft & m): 946 ft (288m)	**Hillslope Profile Position:** Summit	**Geomorph Component:** Interfluve	
Land Cover: None; harvested corn remnants	**Parent Material:** calcareous, loamy glacial till		
Notes / Site Sketch:			



Pedon / Profile Description

Profile/Core: Site 1 – Plowed field **Describer(s): Haley Madden** **Date: 5/2/23**

	Horizon	Depth (cm)	Moist Color	Structure (grade/size/type)	Roots and Pores (Quant./size/location)	Rock and Other Fragments (kind/size/%vol./roundness)
1	Ap1	0-26	10YR 2/1	Strong, coarse, subangular blocky	Corn roots 0-10cm	N/A
2	Ap2	26-47	10YR 3/1	Strong, coarse, subangular blocky	N/A	Some, small
3	Bt1	47-71	10YR 3/2 & 5/2	Strong, coarse, subangular blocky	N/A	Some, small
4	Bt2	71-95	10YR 5/4 with 7.5YR 5/8 redox	Strong, very coarse, subangular blocky	N/A	Many, medium
5						
6						

	Horizon	Boundary (Distinctness)	Consistence	Hand Texture	Misc. Notes (e.g., redox features, concentrations, etc.)
1	Ap1	Abrupt	Firm	Silty clay	Plowed soil; very dark.
2	Ap2	Abrupt	Firm	Silty clay	Very similar to Ap; slightly more clay content and mottles
3	Bt1	Gradual	Firm	Clay loam	Very mottled.
4	Bt2	END	Firm	Clay loam	Gradual transition. More yellow in color. Much redox. Ended due to gravel.
5					
6					

Official Series Description – L113B

Series: Reedslake-Le Sueur (focus on Reedslake)

States soil series located in: MN,

Taxonomic class: Fine-loamy, mixed, superactive, mesic Typic Argiudolls

Typical pedon: Reedslake loam on a convex, south facing 3 percent slope in a cultivated field.

Type Location: Meeker County, Minnesota; about 4.5 miles south of the city of Dassel,

Geographic setting - landform: Reedslake soils are on convex and linear slopes on ground moraines. Slopes range from 2 to 5 percent.

Geographic setting - parent material: They formed in calcareous, loamy glacial till of the Late Wisconsin glaciation.

Mean annual precipitation (mm and in): 635-813; 25-32

Mean annual temperature (C and F): 7.2-10C; 45-50F

Drainage: Well drained (Le Sueur – somewhat poorly drained)

Runoff potential: Moderately low.

Saturated hydraulic conductivity and permeability: Reedslake Permeability is moderate. Reedslake – Capacity of the most limiting layer to transmit water (Ksat): Moderately high to high (0.20 to 2.00 in/hr). Le Sueur permeability is moderate. Capacity of the most limiting layer to transmit water (Ksat): Moderately low to high (0.06 to 2.00 in/hr)

Use and Vegetation: Most areas of these soils are used for cropland. A few areas are in pasture and woodland. Native vegetation is tall grass prairie and mixed northern hardwoods.

Horizon	Depth Range (cm)	Moist Color	Texture	Structure (grade, size, type)	Consistence (friable, firm, loose, etc.)	Boundary
Ap	0-31	Black-10YR 2/1	Loam	weak fine subangular blocky structure	Friable	Abrupt smooth
Bt	31-66	Dark yellowish brown 10YR 4/4	Clay loam	moderate medium subangular blocky structure	Friable	Clear smooth
Cont'd		common very dark grayish brown (10YR 3/2) clay films on faces of peds and in pores, few very dark brown (10YR 2/2) clay films in channels				
Bk1	66-81	Light yellowish brown 2.5Y 6/4	Loam	weak coarse subangular blocky structure parting to weak medium platy	Friable	Clear smooth
Bk2	81-122	Light yellowish brown 2.5Y 6/4	Loam	weak coarse subangular blocky structure	Friable	Clear smooth
Cont'd		few fine distinct light brownish gray (2.5Y 6/2) Fe depletions and few fine prominent strong brown (7.5YR 5/8) Fe concentrations				
C	122-203	Light yellowish brown 2.5Y 6/4	Loam	Massive	Friable	N/A
		common fine distinct light brownish gray (2.5Y 6/2) Fe depletions and few fine prominent strong brown (7.5YR 5/8) Fe concentrations				

Soil Taxonomy Description

Site ID: Site 1_Plowed Field	**Soil Series/Map Unit Name:** L113B: Reedslake-Le Sueur complex, 1 to 6% slopes
Soil Taxonomy: Fine-loamy, mixed, superactive, mesic Typic Argiudolls	
Order formative element and description:	"Olls" for Mollisol
Suborder formative element and description:	"Ud" for Udoll; meaning udic soil moisture regime
Great Group formative element and description:	"Argi" for Argiudoll; Means the soil has an argillic Bt, enriched in illuvial clay
Subgroup formative element and description:	"Typic"; meaning typical moisture expected
Family formative elements (if not given write N/A):	
Texture formative element and description:	"Fine-loamy" meaning the soil has 15% or more particles with diameters of 0.1-75mm & 18-35% clay
Mineral Composition formative element and description: "Mixed"; meaning it does not fit any one minerology class	
Cation Exchange Activity formative element and description: "Superactive"; meaning the soil has a CEC to clay ratio of 0.60 or more	
Soil Temperature Regime formative element and description: "Mesic"; meaning the soil has a mean annual soil temp of 8-15C	
Other Misc. Family formative elements and descriptions: N/A	

Appendix 3. Geomorphic Site Description for Site 2

Site ID: Site 2a_Preferred Forested Area
Date: 5/2/2023
Describers: Haley Madden

Current Weather: Sunny, windy, 52
Past Weather (48 – 72 hours): High 57-58°F (13.8-14.4), Windy, no precipitation

Series / Map Unit Name & Symbol: Lester-Belview complex, 945F; 22 to 40% slope

Taxonomic Classification: Fine-loamy, mixed, superactive, mesic Mollic Hapludalfs

County & State: Nicollet County, MN
Latitude & Longitude (DD or UTM): 44.408075, 94.009158

Land Resource Region: Cropland, pasture, woodland
Major Land Resource Area: 103 – Central and South central MN

Avg Annual Temp (°F & °C): 45-50F; 7.2-10C
Avg Annual Rain (in & cm): 32in; 81cm
Avg Annual Temp (°F & °C): 45-50F; 7.2-10C

Min Avg Temp and Month (°F & °C): -12C; 8.8F; January
Max Avg Temp and Month (°F & °C): 35C; 95F; June

Soil Temperature Regime: Mesic (8C < MAST < 15C)
Soil Moisture Regime: Udic (soil moisture high enough year-round)

Soil Moisture: Moist
Effective Precipitation: Some precipitation stopped by foliage, but north-facing slope and tree coverage also protecting area from direct sunlight, preventing evaporation. On a terrace of a slope with no barrier preventing runoff.

Geomorph Environment: Slope/ Glacial
Landscape: Terrace/Till plain
Landform: Side slope/

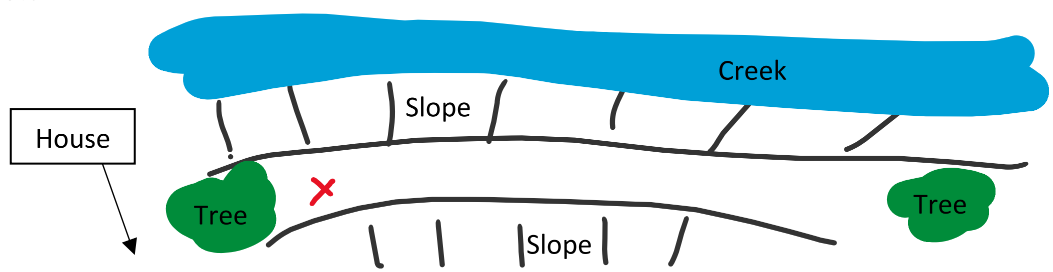
Microfeatures: Situated on a terrace between two mature trees
Anthro Features: House on the summit of the slope. Various debris, garbage, and fencing surrounding the area.

Slope Aspect (°): 0°
Slope Gradient (%): 6-10%
Slope Complexity: Complex
Slope Shape: Convex convex

Elevation (ft & m): 942 ft (287 m)
Hillslope Profile Position: Footslope
Geomorph Component: Side slope

Land Cover: Hardwood forest (oak, elm, etc.). Leaf litter on forest floor.
Parent Material: calcareous, loamy glacial till, late Wisconsinan Age.

Notes / Site Sketch:



Pedon / Profile Description

Profile/Core: Site 2 – Forest **Describer(s):** Haley Madden **Date:** 5/2/23

	Horizon	Depth (cm)	Moist Color	Structure (grade/size/type)	Roots and Pores (Quant./size/location)	Rock and Other Fragments (kind/size/%vol./roundness)
1	A	0-34	10YR 3/1	Moderate, medium, subangular blocky	Common, fine, throughout	N/A
2	AB	34-74	10YR 3/1	Moderate, medium, subangular blocky	Common, fine, throughout	N/A
3	Bt	74-104	10YR 3/2	Moderate, medium, subangular blocky	Moderately few, coarse, common	N/A
4	Bt2	104-120	10YR 3/2	Moderate, medium, subangular blocky	Moderately few, coarse, towards surface	Some rocks/ gravel; evidence of glacial till
5						

	Horizon	Boundary (Distinctness)	Consistence	Hand Texture	Misc. Notes (e.g., redox features, concentrations, etc.)
1	A	Gradual	Friable	Silty clay	No clear boundaries between horizons
2	AB	Gradual	Friable	Silty clay	No evidence of calcium concretions in subsoils.
3	Bt	Gradual	Friable	Clay loam	
4	Bt2	Gradual	Friable	Silty clay	Increase in gravel could indicate nearing glacial till/ C horizon with depth
5					

Official Series Description – 945F

Series: Lester-Belview complex (focus on Lester)	**States soil series located in:** MN, IA
Taxonomic class: Fine-loamy, mixed, superactive, mesic Mollic Hapludalfs	
Typical pedon: Lester loam, on a convex slope of about 9 percent, on a ground moraine, in a cultivated field.	
Type Location: 103-Central Iowa and Minnesota Till Prairies, Wright County, Minnesota subset	
Geographic setting - landform: convex slopes on moraines and till plains.	
Geographic setting - parent material: calcareous, loamy till, late Wisconsinan Age.	
Mean annual precipitation (mm and in): 585-890; 23-35	**Mean annual temperature (C and F):** 6-10C; 43-50F
Drainage: Well drained (Belview: well)	**Runoff potential:** Moderately low to high
Saturated hydraulic conductivity and permeability: Lester – Moderately permeable; Capacity of the most limiting layer to transmit water (Ksat): Moderately high to high (0.20 to 2.00 in/hr). Belview – Moderately high to high (0.20 to 2.00 in/hr)	
Use and Vegetation: Most areas are cultivated. The principal crops are corn and soybeans. Some areas are pastureland and forest. The native vegetation is savanna	

Horizon	***Depth Range (cm)***	***Moist Color***	***Texture***	***Structure (grade, size, type)***	***Consistence (friable, firm, loose, etc.)***	***Boundary***
Ap	0-18	Very dark grayish brown 10YR 3/2	Loam	moderate fine subangular blocky structure	Friable	Abrupt smooth
Bt1	18-53	Brown (10YR 4/3)	Clay loam	moderate medium subangular blocky structure	Firm	Clear smooth
Bt2	53-97	dark yellowish brown (10YR 4/4)	Clay loam	moderate medium subangular blocky structure	Friable	Clear smooth
Bk1	97-127	yellowish brown (10YR 5/4)	Loam	weak medium subangular blocky structure	Friable	Clear wavy
Bk2	127-152	yellowish brown (10YR 5/4)	Loam	weak medium subangular blocky structure	Friable	Clear wavy
C	152-203	Yellowish brown (10YR 5/4)	Loam	Massive	Friable	N/A

Soil Taxonomy Description

Site ID: Site 2a_Preferred Forested Area	**Soil Series/Map Unit Name:** 945F: Lester-Belview complex, 22 to 40
Soil Taxonomy: Fine-loamy, mixed, superactive, mesic Mollic Hapludalfs	
rder formative element and description:	"Alf" for Alfsols; Soils, usually less acidic than Ultisols and in cooler climates, that have a Bt horizon enriched in silicate clays, or a fragipan
Suborder formative element and description:	"Ud" for Udoll; meaning udic soil moisture regime (humid, moist)
Great Group formative element and description:	"Hapl" for Haplic; minimum horizonation, not a "complicated" profile
Subgroup formative element and description:	"Mollic" meaning Intergraded to a Mollisol, often implying that A horizon is not quite dark and/or thick enough to be a mollic epipedon.
Family formative elements (if not given write N/A):	
Texture formative element and description:	"Fine-loamy" meaning the soil has 15% or more particles with diameters of 0.1-75mm & 18-35% clay
Mineral Composition formative element and description: "Mixed"; meaning it does not fit any one minerology class	
Cation Exchange Activity formative element and description: "Superactive"; meaning the soil has a CEC to clay ratio of 0.60 or more	
Soil Temperature Regime formative element and description: "Mesic"; meaning the soil has a mean annual soil temp of 8-15C	
Other Misc. Family formative elements and descriptions: N/A	

Appendix 4: Weight of the pig samples, before and after decomposition

	Frozen weight (g)		Decomposed weight (g)		Weight lost (g)		Percentage of original mass	
	Site 1	*Site 2*	*Site 1*	*Site 2*	*Site 1*	*Site 2*	*Site 1*	*Site 2*
	387	474	96	186	291	288	24.8%	39.2%
	468	406	131	124	337	282	28.0%	30.5%
	383	354	90	81	293	273	23.5%	22.9%
Averages	412.7	411.3	105.7	130.3	307	281	25.4%	30.9%
Averages in group	412		118		294		28.2%	

Appendix 5. Close-up of invertebrates on a sample from Site 1

Appendix 6. Pig sample 1.2, post-decomposition. Fewer mites are present, but more fungus is growing directly on the bone. More adipocere (far left) was also present than on sample 1.1.

Appendix 7. Close-up of pig sample 1.2, post-decomposition. View of compact bone decay.

Appendix 8. Pig sample 1.3. Little to no invertebrate activity was observed and less adipocere tissue than sample 1.2 had formed.

Appendix 9. Pig sample 2.2. Soil and roots were pulled from around the bones during cleaning. The bones showed signs of fungus but little to no invertebrate activity.

Appendix 10. Pig sample 2.3. This sample was partially damaged during excavation and the metatarsals were missing. This was the only sample that had green mold present on the adipocere tissue.

Table 1. EARTH Lab Analysis

Averages of EARTH Lab Analysis												
	10th Percentile	*Median*	*90th Percentile*									
Sample	**Dx (10)**	**Dx (50)**	**Dx (90)**	**%Clay**	**%Silt**	**%Sand**	**Adjusted %Clay***	**Adjusted %Silt***	**%Sand**	**Adjusted %Silt+ Clay****	**Adjusted %Sand+ Silt+ Clay****	**Soil Type**
Site 1	1.59	8.55	34.92	28.78	67.54	3.68	48.00	48.32	3.68	96.32	100.00	silty clay
Site 1	1.60	8.71	37.08	28.43	66.92	4.64	47.44	47.92	4.64	95.36	100.00	silty clay
Site 1	1.59	8.58	35.09	28.70	67.59	3.71	47.87	48.42	3.71	96.29	100.00	silty clay
Site 2	1.88	10.96	55.79	23.97	67.06	8.97	41.12	49.91	8.97	91.03	100.00	silty clay
Site 2	1.87	10.90	53.89	24.04	67.37	8.59	41.24	50.17	8.59	91.41	100.00	silty clay
Site 2	1.88	11.03	56.69	23.85	67.04	9.10	40.93	49.97	9.10	90.90	100.00	silty clay
Average of Site 1	1.59	8.61	35.64	28.64	67.35	4.01	47.77	48.22	4.01	95.99	100.00	silty clay
Average of Site 2	1.88	10.96	55.46	23.95	67.16	8.89	41.10	50.02	8.89	91.11	100.00	silty clay

Percentage of Each Fraction												
	Colloid	*Clay*	*Very Fine Silt*	*Fine Silt*	*Medium Silt*	*Coarse Silt*	*Very Fine Sand*	*Fine Sand*	*Medium Sand*	*Coarse Sand*	*Very Coarse Sand*	*Pebble**
Sample	**0.01-0.25 µm**	**0.25-4 µm**	**4-8 µm**	**8-16 µm**	**16-31 µm**	**31-62 µm**	**62-125 µm**	**125-250 µm**	**250-500 µm**	**500-1000 µm**	**1000-2000 µm**	**2000-3500 µm**
Site 1	0.00	28.78	19.22	21.83	17.95	8.55	2.89	0.78	0.00	0.00	0.00	0.00
Site 1	0.00	28.43	19.01	21.60	17.79	8.53	2.88	0.78	0.62	0.35	0.00	0.00
Site 1	0.00	28.70	19.17	21.85	17.96	8.61	2.92	0.79	0.00	0.00	0.00	0.00
Site 2	0.00	23.97	17.15	20.37	18.86	10.68	4.50	2.61	1.69	0.17	0.00	0.00
Site 2	0.00	24.04	17.20	20.47	18.97	10.73	4.65	2.65	1.27	0.02	0.00	0.00
Site 2	0.00	23.85	17.08	20.35	18.78	10.84	4.79	2.77	1.52	0.03	0.00	0.00
Average of Site 1	0.00	28.64	19.13	21.76	17.90	8.56	2.90	0.78	0.21	0.12	0.00	0.00
Average of Site 2	0.00	23.95	17.14	20.40	18.87	10.75	4.64	2.68	1.49	0.07	0.00	0.00

* %Clay is underestimated and %Silt is overestimated due to the principles of laser defractometry (Eshel et al., 2004; Buurman et al., 2001; Konert and Vandenberghe, 1997). To account for this underestimation of the clay fraction, a series of experiments were conducted and results indicate that including the "Very Fine Silt" fraction with the clay fraction greatly improves results (Bowen, 2022). For more information on these experiments you can contact Mark Bowen at mark.bowen@mnsu.edu.

Table 2. Ward Lab Analysis

Site	When sample was taken	1:1 Soil pH	WDRF Buffer pH	1:1 Soluble Salts mmho/cm	Organic Matter LOI %	KCl Nitrate ppm N	Nitrate lbs (N per Acre)	Ammonium Acetate Extractable Cations (What is available to plants)				Sulfate-S ppm S
								Potassium ppm K	Calcium ppm Ca	Magnesium ppm Mg	Sodium ppm Na	
Site 1 Crop Soil	*Pre-Decomposition*	6.7	7.2	0.21	5.5	22.2	80	99	3191	672	11	5.5
	Post - Decomposition	5.7	6.8	0.55	5	96	346	67	2402	489	22	12.8
Site 2 Forest Soil	*Pre-Decomposition*	6.2	6.7	0.44	7	62.1	224	438	2582	485	22	7.3
	Post - Decomposition	6.7	7.2	0.26	6.1	31	112	438	2541	461	27	8.3

Site	When sample was taken	DTPA (Extractant for the chemicals)				CEC/Sum of Cations me/100g	Phosphorus ppm	% Base Saturation				
		Zinc ppm Zn	Iron ppm Fe	Manganese ppm	Copper ppm Cu			%H Sat	%K Sat	%Ca Sat	%Mg Sat	%Na Sat
Site 1 Crop Soil	*Pre-Decomposition*	0.55	32.8	11.6	*0.77*	21.9	10	0	1	73	26	0
	Post - Decomposition	0.52	26.9	30.8	*0.71*	18.2	38	10	1	66	22	1
Site 2 Forest Soil	*Pre-Decomposition*	3.53	94.3	5	*0.85*	20.8	124	13	5	62	19	0
	Post - Decomposition	3.64	57.7	7.5	*0.91*	17.8	149	0	6	71	22	1

Table 3. Megyesi categories and stages of decomposition for the limbs

Stage	Points	Description
Fresh	1	Fresh, no discoloration
Early Decomposition	2	Pink-white appearance with skin slippage of hands and/or feet.
	3	Gray to green discoloration; marbling; some flesh still relatively fresh.
	4	Discoloration and/or brownish shades particularly at edges, drying of fingers, toes, and other projecting extremities.
	5	Brown to black discoloration, skin having a leathery appearance.
Advanced Decomposition	6	Moist decomposition with bone exposure less than one half that of the area being scored.
	7	Mummification with bone exposure of less than one half that of the area being scored.
Skeletonization	8	Bone exposure over one half the area being scored, some decomposed tissue and body fluids remaining.
	9	Bones largely dry, but retaining some grease.
	10	Dry bone.

Table 4. Temperature, accumulated degree days (ADD), and precipitation data during the experiment. Averages and sums were taken for brevity.

Month	Max Temp *Average* (°F)	Min Temp *Average* (°F)	ADD *Average* (°F)	Max Temp *Average* (°C)	Min Temp *Average* (°C)	ADD *Average* (°C)	Precipitation (inches) *Average*	Precipitation (inches) *Total*
May	74.06	48.8	61.43	23.37	9.33	16.35	0.02	5.87
June	83.86	58.97	71.42	28.81	14.98	21.89	0.04	1.17
July	82.29	57.48	69.89	27.94	14.16	21.05	0.07	1.89
August	81.74	60.03	70.89	27.63	15.57	21.60	0.16	4.65
September	76.63	53.97	65.30	24.79	12.20	18.50	0.09	2.58
October	73.13	52.62	62.88	22.85	11.46	17.15	0.17	0.17
Averages	78.62	55.31	N/A	25.90	12.95	N/A	0.09	2.72
Totals	N/A	N/A	11151.87	N/A	N/A	3262.15	0.57	16.33

Table 5. Descriptions of pig remains, pre- and post-decomposition.

	Pre-Decomposition		**Post-Decomposition**						
Sample	*Weight (g)*	*Description*	*Weight (g)*	*Any remaining flesh*	*Invertebrate activity*	*Fungal & Faunal Activity*	*Bone color*	*Bone condition*	*Adipocere*
1.1	387	Pink muscle tissue, pale pink skin, and cut bone observed. No discoloration, decay, floral, or faunal activity.	96	Some flesh and hair present on the anterior side of the tarsal.	About 10 mites per cm2and 1 springtail per cm2. Brown spiders and pot worms observed.	Yellow fungus in cut end of bone. No major root growth observed.	Black (5YR 2.5/1), white (7.5YR 8/1), dark gray (10YR 4/1), and dark brown (10YR 3/3) with shades varying in between.	Bones largely intact. Still fresh/greasy.	Some adipocere present (about 12 x 7cm)
1.2	468		131	None.	About 7 mites per cm2and 1 springtail per cm2. Brown spiders and pot worms observed.	White fungus covering much of the long bones. No major root growth observed.		Bones largely intact. Still fresh/greasy, with some compact bone decay.	Some adipocere present (about 10cm diameter)
1.3	383		90	None.	No springtails or mites. Some brown spiders and pot worms observed.	Some white and yellow fungus on distal ends of cut long bones. No major root growth observed.		Bones largely intact. Still fresh/greasy.	Some adipocere present (about 5 x 10cm)
2.1	474		186	Some hairs present on the distal end of the calcaneus.	Some small larvae observed.	White fungus covering much of the bones. Roots completely encased the bones in the space between the adipocere.	Black (5YR 2.5/1), dark brown (10YR 3/3). Dark reddish brown (2.5YR 3/4), and pale brown (2.5Y 8/4) observed on adipocere tissue.	Bones not as noticeably greasy. Some compact bone decay observed.	The most adipocere observed (about 10 x 20 cm on one side) . Completely surrounding roots and bones.
2.2	406		124	None.	Little to no invertebrate activity.	White fungus covering much of the bones. Roots completely encased the bones in the space between the adipocere.		Bones not as noticeably greasy. Some compact bone decay observed.	Large pieces of adipocere present (about 15 x 10cm) but much fused to the surrounding soil and destroyed during cleaning.
2.3	354		81	None.	Little to no invertebrate activity.	Some fungus observed on bones. Green mold present on exterior of adipocere tissue.		Bones noticeably greasy. One bone partially damaged during excavation and missing metatarsals.	Large strip of fungus present (about 20 x 5cm). Some lost during excavation.