## TERMITE MOUND SOIL AS A SOIL AMENDMENT IN EASTERN UGANDA

## A Thesis

by

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## MASTER OF SCIENCE

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#### ABSTRACT

Food scarcity in Sub-Saharan Africa can be attributed in large part to the degradation of soils, due to factors such as soil nutrient and organic matter depletion. Regardless of how efficiently any other possible factors are handled, yield thresholds will continue to decline until soil fertility is addressed. The lack of affordable inorganic fertilizer is a large contributor to this problem. Therefore, the use of organic amendments such as manures and composts are commonly recommended as an alternative to farmers in rural communities. However, the availability and affordability of these amendments is frequently limited, leaving few remaining alternatives to the ongoing degradation of soil productivity.

The purpose of this study is to determine the effectiveness of applying *Macrotermes* soil as an organic amendment to initiate a soil restoration process that is more financially accessible. These termites build their structures out of subsurface clay particles, which have been reported to contain significantly higher concentrations of plant-available nutrients than the surrounding topsoil. These mounds are a public resource, meaning that anyone who lives near a mound will have access to the material. Mounds are treated as a nuisance by many people, who dispose of the mound soil away from their fields, thereby wasting any potential benefit.

This study will be conducted in Kyando, Uganda, over one growing season. The effects of both living and dead termite mound soil on maize (*Zea mays*) yield and arbuscular mycorrhizal fungi association with corn roots will be compared to maize treated with cattle manure and maize receiving no amendment. A chemical analysis will

also be performed on a survey of *Macrotermes* mounds in the area. These results are expected to support the development of an improved management strategy that can assist disadvantaged farmers in East Africa in the restoration of their soil fertility, increase yields, and taking a first step towards alleviating food insecurity.

## **DEDICATION**

Dedicated to my husband, Bryan. Thank you for everything you've done throughout my degree, from accompanying me on 2 am lab visits to going to Uganda with me to help with my research. I love you so much and couldn't have finished this without you.

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#### **Contributors**

This work was supervised by a thesis committee consisting of Dr. Mowrer of the Department of Soil and Crop sciences, Dr. Provin of the Department of Soil and Crop Sciences, and Dr. Murano of the Borlaug Institute.

The Fertility data analyzed for chapters 3 and 4 was completed by Jabba Engineering Ltd. in K'la, Uganda. All other work conducted for the thesis was completed by the student independently.

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## NOMENCLATURE

SSA Sub-Saharan Africa

N Nitrogen

P Phosphorus

K Potassium

FOB Free on Board

NARO National Agricultural Research Organization

FAO Food and Agriculture Organization

NGO Non-Government Organization

XRD X-ray Diffraction

DRC Democratic Republic of the Congo

EC Electrolytic Conductivity

DI Deionized

TDS Total Dissolved Solids

AMF Arbuscular Mycorrhizal Fungi

KOH Potassium Hydroxide

ANOVA Analysis of Variance

LSD Least Significant Difference

BMP Best Management Practice

## TABLE OF CONTENTS

	Page
ABSTRACT	ii
DEDICATION	iv
ACKNOWLEDGEMENTS	V
CONTRIBUTORS AND FUNDING SOURCES	vi
NOMENCLATURE	vii
TABLE OF CONTENTS	viii
LIST OF FIGURES	X
LIST OF TABLES	xii
1. INTRODUCTION	1
1.1. References	3
2. REVIEW	6
2.1. Soil Degradation in East Africa  2.2. Agricultural Inputs  2.3. Termite Mound Soil as an Agricultural Input  2.4. Arbuscular Mycorrhizal Fungi  2.4.1. Importance to Smallholder Agriculture  2.5. Interactions between Termite Mound Soil and AMF.  2.6 References	9 35 42
3. EVALUATION OF MACROTERMES MOUND SOIL AS A SOIL AMENDMENT	63
3.1. Introduction	68 68 69
3.2.4. Statistical Analysis  3.3. Results and Discussion  3.3.1. Physical Survey	78

3.3.2. Chemical Survey	84
3.3.3. Potential as Amendment	
3.4. Conclusions	
3.5. References	108
4. APPLICATION OF MACROTERMES MOUND SOIL AS A SOIL	
AMENDMENT FOR MAIZE IN EASTERN UGANDA	112
4.1. Introduction	112
4.2. Methods	
4.2.1. Study Site	
4.2.2. Field Trial	
4.2.3. Data Collection	
4.2.4. Arbuscular Mycorrhizal Fungi (AMF)	126
4.2.5. Statistical Analysis	
4.3. Results and Discussion	128
4.3.1. Field Chemistry	128
4.3.2. Yield	
4.3.3. Arbuscular Mycorrhizal Fungi	
4.3.4. Implications for Farmers	
4.4. Conclusions	
4.5. References	149
5 CONCLUSIONS	153

# LIST OF FIGURES

Page
Figure 2.1 Examples of fungus galleries containing <i>Termitomyces</i> combs inside of two different <i>Macrotermes</i> mounds in Kyando, Uganda. Lighter colored, Nenriched nodules are visible on top of combs pictured on the right. Images photographed at the study site by Kimberlyn M. Pace
Figure 2.2 Mycorrhizal structures observed colonizing maize roots ( <i>Zea mays</i> ) in Kyando, Uganda. Roots were cleared with potassium hydroxide and photographed by Kimberlyn M. Pace under 10x magnification using an Iphone 7 camera. (A) An arbuscule, (B) a cluster of vesicles, (C) two vesicles (white circle), an arbuscule (black arrow), hyphae (white arrow), and (D) extracellular hyphae (black arrow) and an external vesicle (white arrow).
Figure 2.3 Differences in hyphal thickness observed between two maize ( <i>Zea mays</i> ) root samples in Kyando, Uganda. Roots were cleared with potassium hydroxide and photographed by Kimberlyn M. Pace under 10x magnification
Figure 3.1 Map displaying the location of all <i>Macrotermes</i> mounds located at the 22-acre Vocare Ministries Farm in Kyando, Uganda. The map was created using Google Maps and GPS coordinates from a handheld Garmin GPS70
Figure 3.2 Examples of a <i>Macrotermes</i> mound from each vegetation rank, photographed at the study site by Kimberlyn M. Pace with a 30 cm ruler for scale. (A) rank 1, (B) rank 2, (C) rank 3, and (D) rank 4
Figure 3.3 Examples of a <i>Macrotermes</i> mound from each erosion rank, photographed at the study site by Kimberlyn M. Pace, with a 30 cm ruler for scale. (A) rank 1, (B) rank 2, (C) rank 3, and (D) rank 4
Figure 3.4 Distribution of NPK by mound depth at half-height, comparing a living <i>Macrotermes</i> mound to a dead one. Samples were taken from four depths, 0-15 cm (M 0"), 15-30 cm (M 6"), 30-45 cm (M 12"), and 45-60 cm (M 18"). Values on bars represent nutrient concentrations in each sampling depth in mg kg <sup>-1</sup> .
Figure 3.5 Distribution of NPK in the outer wall of a living <i>Macrotermes</i> mound compared to a dead one. Samples were taken from the bottom 0-30 cm layer (B 0"), middle 0-15 cm layer (M 0"), and top 0-15 cm layer (T 0"). Values on bars represent nutrient concentrations in each sampling depth in mg kg <sup>-1</sup> 99

Figure 4.1 This diagram represents soil core locations as well as the estimate of the location of the mound, where (o) represents a soil texture change in the 15-30 cm soil layer from sandy loam to sandy clay loam, indicative of the mound presence, and (x) represents where the soil texture represented that of the surrounding sandy loam topsoil	22
Figure 4.2 Total maize grain (kg) and biomass (kg) yield observed per treatment.  Grain yield includes field-dry kernels which had been ginned. Biomass yield includes empty husks and stalk tissue. Standard deviation bars are included to represent deviation between treatments.	35
Figure 4.3 This figure displays dry grain mass (g) per cob post-ginning, averaged by treatment. These values represent field-dry maize kernels which were removed from the cob before being weighed. Standard deviation bars are included to represent deviation between treatments.	35
Figure 4.4 Examples of root architecture resulting from different amendment added.  (A) Control, (B) live termite mound soil, (C) dead termite mound soil, (D) manure. The roots were photographed at the study site by Kimberlyn M.  Pace	39
Figure 4.5 Average arbuscules and average vesicles observed per treatment.  Structures were observed under 10x magnification. Standard deviation bars are included to represent deviation between treatments	12
Figure 4.6 Average AMF percent by treatment, calculated using (Eq. 2). Standard deviation bars are included to represent deviation between treatments14	13

# LIST OF TABLES

Page
Table 3.1 Description of Mound Vegetation Ranking System
Table 3.2 Description of Mound Erosion Ranking System
Table 3.3 Physical Survey Results
Table 3.4 Volume (m³) of Living and Dead Mounds by Landscape Position81
Table 3.5 Chemical Survey Results
Table 3.6 Spatial Survey Results
Table 3.7 Fertility by Overall Mound Size Regardless of Status
Table 3.8 Fertility Results of Mound Size by Status Analysis95
Table 3.9 Standard Deviations of Mound Size by Status Data (Table 3.7)96
Table 3.10 NPK Comparison of <i>Macrotermes</i> Outer Wall to Surrounding Topsoil100
Table 3.11 Potential of 34 <i>Macrotermes</i> Mounds as a Fertility Amendment
Table 4.1 NPK Added as Amendment in the Field Trial
Table 4.2 Ranking System Used to Describe AMF Colonization of Maize Roots 127
Table 4.3 NPK Values Recorded per Plot. 129
Table 4.4 pH and EC Values Recorded per Plot
Table 4.5 Field pH and EC Values Averaged by Treatment
Table 4.6 Field Trial Yield Results. 133
Table 4.7 AMF Field Trial Results

#### 1. INTRODUCTION

There are an estimated 254 million food insecure people in Sub-Saharan Africa (SSA), and this number is expected to rise in the coming decade due to pressures from increasing populations (Ittersum, 2016; Rosen, 2013; Tittonell, 2013) and urbanization without agricultural growth (Djurfeldt, 2015). Roughly 33 % of people in SSA are classified as undernourished, with greater than 60 % of these people living in East Africa (Khan, 2014). Long term food security cannot be achieved without first supporting and empowering smallholder farmers to be more self-sufficient (WDR, 2008). Smallholder farmers, also commonly referred to as subsistence farmers, are generally rural farmers whose predominant source of income is agricultural production on small, low-input, family-run farms which do not sell a majority of their produce to public markets (Barnett, 1997; Cornish, 1998; Morton, 2007). These farmers constitute the majority of the food production capacity of the region (Adamtey, 2016; Frelat, 2016; Herrero, 2010) yet they have a grain self-sufficiency ratio (food produced to food imported) of 0.8, one of the lowest in the world (Ittersum, 2016). Though there have been measurable cereal grain yield increases, these have not kept up with the rate of population increase, necessitating the importation of food (Ittersum, 2016). Much of SSA has since reverted back to being a net importer of grain instead of a net exporter (Khan, 2014).

A major component of the second Green Revolution, or change(s) in agricultural production necessary to feed and sustain the Earth's growing population, is anticipated

be the introduction of nutrient stress tolerant plants that can thrive in depleted soils to close these yield gaps, causing a significant sum of money to be invested in such projects (Lynch, 2007). However, widespread adoption of new varieties is unlikely due to the expense of hybrid seed and the mechanized processes that often accompany their inclusion into practice (Dawson, 2016). In addition, there are often political and economic failures which contribute to low adoption rates, as well as lack of breeding development on local root crop staples compared to global staples (Evenson, 2003). In a study conducted to quantify the potential benefit of improved cultivars, improved crop varieties only accounted for 28 % of yield increases in SSA compared to 70-90 % of yield increases in Asia and Latin America (Sánchez, 2005). This disparity is attributed to the severe degradation of soil fertility observed throughout the African continent. Between 1960 and 2000, Asia and Latin America have seen their food production triple while a large portion of SSA still struggles to produce enough to survive (Sánchez, 2010). From 1990-2010 maize yields in SSA have increased from around 1.4 tons per ha to 1.8 tons, but this remains near the global base of reported maize yields (FAO 2012). There is a need to develop a new approach to address the overall decline in food production per capita that farmers in many parts of Africa are experiencing.

There are many inter-related causes of the declining agricultural production observed in SSA, such as lack of infrastructure and economic growth, pressure from insect pests, soil nutrient depletion, and degraded soils (Adamtey, 2016; Folberth, 2014; Henao, 2006; Khan, 2014). This thesis explores a short-term solution to the general decline in soil fertility by focusing in on, and providing improvements in soil

management, to urgently and critically failing small landholder systems. Specifically, *Macrotermes* soil is examined as an alternative to commonly recommended organic amendments in low-input systems. A survey and field experiment were conducted to develop a management strategy that uses termite mound soil to improve small landholder economic outcomes in the short term, enabling them to build resources to a point that facilitates integration of livestock and manure resources.

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#### 2. REVIEW

#### 2.1. Soil Degradation in East Africa

The biggest cause of the widespread infertility observed throughout SSA is the failure to replace nutrients lost to centuries of cropping, following a shift from slash and burn agriculture to permanent cultivation (Bedada, 2014; Henao, 2006; Khan, 2014; Kihara, 2016; Sileshi, 2010a). Of the inputs being added back to fields, the amounts are too small to replenish what is taken away each year (Bedada, 2014). Across SSA, nitrogen (N), carbon, and phosphorus (P) are the three most common nutrient deficiencies observed in these soils. N and P are essential to crop production and therefore have negative effects on crop yields when present in low amounts, whereas lack of carbon as soil organic matter effectively stunts microbial activity in soils (Sánchez, 2002) which in turn impacts nutrient mineralization. Lack of organic carbon also decreases aggregate stability and water retention in soils. There are especially low levels of available P in the arable soils of East Africa, with 80 % of the land owned by small scale maize farmers classified as "extremely deficient" (Sánchez, 2002). These soils are characterized by high rates of P fixation via sorption to aluminum and iron hydroxides, making it unavailable to plants (Cardoso, 2006). The problem continues to worsen due to lack of P-fertilizer inputs to replace losses of plant available soil-P. The net effect is that soils are becoming more nutrient depleted and less productive every year.

The soils of East Africa are most commonly classified as Oxisols, the highly weathered soils of the tropics. Compounding the unsustainable agricultural practices common throughout the region such as mono-cropping, these soils typically have low native fertility due to their low cation exchange

capacity and high anion adsorption by iron and aluminum oxides. For example, Jama (1999) recorded values below 5 mg kg<sup>-1</sup> available P in soils located in Western Kenya that had a P sorption capacity of 300 mg P kg<sup>-1</sup> (calculated by Nziguheba, 2001).

Heavy tropical rains common throughout this region hasten the weathering and leaching of these soils further. This allows for the loss of plant essential nutrients such as N and K. P is not typically a leached mineral due to low overall solubility but it can be eroded along with loose topsoil or otherwise weathered by climate processes. Much of the P remaining in the soil profile is often located outside of the depth which is accessible to plant roots, due to a lack of inputs at the surface of the soil. Lands are over grazed and extremely low in organic matter, as many farmers do not have the proper equipment to collect and incorporate organic residue back into their fields.

Because of increased population pressures and the continual increase in food production per acre, nutrients are being depleted at an exponential rate from soils which are already predisposed to limit plant access to nutrients. Stoorvogel and Smaling (1990) concluded that roughly 200 million ha of crop land in SSA have lost 660 kg N ha<sup>-1</sup>, 75 kg P ha<sup>-1</sup>, and 450 kg K ha<sup>-1</sup> from the 1960's through the 1990's primarily through the removal of crop harvests without re-incorporating the nutrients from the crop tissue (Sánchez 1997). More recent numbers indicate that the average annual depletion rate for 37 countries in SSA is around 22 kg N, 2.5 kg P, and 15 kg K ha<sup>-1</sup> (Smaling 1997). In Uganda specifically, N balances are much greater at -48.02 kg ha<sup>-1</sup>, P as -10.8 kg ha<sup>-1</sup>, and K as -51.09 kg ha<sup>-1</sup> (Nkonya, 2005). This depletion leads to soil degradation and food insecurity throughout many parts of SSA (Quiñones, 1997; Sánchez 2002; Scherr, 2000;

Tittonell, 2013), a financial loss of around \$4 billion USD per year in fertilizer (Sánchez 2002).

Nutrient mining is a leading cause of soil degradation observed in Uganda, to the point where food production has been steadily dropping despite an overall increase in cultivated land. In fact, only 5 % of Ugandan households had a positive macronutrient balance, indicating that 95 % of households in Uganda were removing more nutrients than were returning to the soil (Nkonya, 2005).

"Soil capital" is a term which can be used to denote the health and area of land which an individual farmer owns (Marenya, 2007). This resource is of the upmost importance to subsistence farmers who depend on it for their livelihoods. The lush vegetation present in this region was originally interpreted as an indicator of soil fertility before it was clear that fertility needed to be replenished. This led to the clearing and over cultivation of much of the land (Zake, 1999). When natural nutrient cycling is disrupted, such as with the lush forests and savannas of this region, soils are no longer able to naturally replenish themselves as they once were. The soils were in fact very fertile, but only because of highly effective nutrient cycling balances which were thrown out of balance with the addition of cultivated crops, especially maize.

The soils found throughout Eastern Uganda are often very productive for agriculture if properly taken care of, but because of the lack of inputs and other unsustainable land management practices, many of these fields are hardly producing enough for families to sustain themselves (Amsalu, 2007; Bedada 2014). Specifically, observations include decreased soil organic matter content, decreased the ability of

microorganisms to recycle nutrients, and reduced the soil's water holding capacity, among other adverse effects (Sánchez, 2005).

## 2.2. Agricultural Inputs

These issues could be addressed with moderate application of mineral fertilizers, but many countries throughout SSA cannot economically justify inorganic fertilizer manufacturing facilities and infrastructure due to low domestic demand (Quiñones, 1997; Sánchez, 2005). Local manufacture and purchase of imported fertilizer are both cost prohibitive (Bumb, 2011; Kihara, 2016). For example, Uganda has significant rock P reserves, but the country does not have adequate infrastructure to refine it or distribute it to their own population (Okalebo, 2007; Sánchez, 2002). Successful manufacture of P fertilizer is often paired with access to substantial sulfur (S) or hydrochloric acid (HCl), an important reagent in the refining process, within the country. While Uganda does have several potential S reserves, they have not been investigated with the intention of mining (Mathers, 1994). There is potential for S mining with the intent of NPK fertilizer manufacture in the future however the infrastructure and logistics are not there yet. This makes it difficult to refine the low-grade P deposits found within the country, increasing reliance on imports.

Transportation infrastructure, or lack thereof, is also a contributor. A majority of the subsistence farmers which are most in need of fertilizers are located far away from major city centers and are very spread out geographically with no way of transporting themselves to large markets (Bedada, 2014). Roads are also in varying condition and are

unsuitable for reliable and inexpensive transportation from borders and major cities. A majority of trucks, parts, and repair equipment must be imported with high import tax to many countries which lack auto manufacturing infrastructure. This increases distribution costs for import companies and often leads to older trucks being used, which are less reliable, especially on poor roads. When mineral fertilizers do end up in rural stores, it is often only urea, and in very small quantities. P, K, and other fertilizers (especially micronutrients) are rarely found in many rural communities (personal communication).

By the time that imported fertilizer reaches individual farmers, it can be anywhere between 2 and 6 times more expensive than it would be if purchased in North America or Europe (Donovan, 1996), depending on how far away the country is from the place of initial import. In the case of Uganda, a landlocked country, most imports are first received in Kenyan ports and then transported over land before they are imported again at the Ugandan border. Spot checks in 2002 revealed that a metric ton of Urea costs around US \$90 free on board (FOB) in Europe, \$120 delivered to the port in Mombasa, Kenya, \$400 in Western Kenya (overland transport), \$500 after crossing the Kenyan-Ugandan border, and \$770 in Malawi after being transported from Beira (Sánchez 2002). The price of a metric ton of Urea has risen to roughly \$260 USD as of January 2019 (IndexMundi, 2019), almost tripling the costs at every single point of transfer. Because of these reasons, application of inorganic fertilizers in East Africa is on average too low to replace nutrients lost to agronomic processes because subsistence farmers cannot afford to replace nutrients with inorganic fertilizers at the recommended rates (Karltun, 2013). A majority of the inorganic fertilizers being imported are

purchased by large plantations for use on cash crops such as sugarcane, tobacco, coffee, and tea (Scheinemachers, 2007).

In Uganda, where 40 % of their overall GDP relies on agriculture (Eilu, 2003), a majority of these fertilized crops are being exported, generating 90 % of the country's foreign exchange earnings (NEMA, 1998). This creates little incentive to make inorganic fertilizers more accessible to subsistence farmers who are not exporting their farm products and are instead consuming them within the farm. As of 2007, Uganda had a rate of 2 kg inorganic fertilizer applied per hectare (FAO 2007), where only 10 % of smallholder farms were observed applying any at all (Pender, 2001). The National Agricultural Research Organization (NARO) and the Food and Agricultural Organization (FAO) estimate from 1999 claim that the mineral NPK application rate in Uganda is even lower at 1 kg ha<sup>-1</sup> (NARO, FAO 1999). The slight increase in application rate from 1999 to 2007 may result from increased application by larger plantations, because a majority of smallholder farmers do not even have access to purchase inorganic fertilizers in many places. However, at rates this low, it is likely that there is no fertilizer application at all for a majority of these farms, as a rate of 1 kg ha<sup>-1</sup> is not easily quantifiable in many of these rural communities. Compared to the overall smallholder farm application rate of N fertilizer averaged over all of SSA, which is 13 kg ha<sup>-1</sup> (Njoroge, 2017), Ugandans are applying some of the lowest rates of inorganic fertilizer in SSA. Average maize yields are also a reflection of these values; on average, rain fed maize yields in SSA fall between 1.2-2.2 tons ha<sup>-1</sup>, 15-27 % of the total potential yield (Njoroge, 2017), whereas maize yields from East Ugandan farms surrounding the study

site were as low as 350 kg ha<sup>-1</sup> (personal communication), which is around a quarter of the overall average for SSA.

These issues are compounded because it is also difficult for many subsistence farmers to obtain credit (IFPRI, 2010), barring most from any type of investment in cattle, inorganic fertilizers, improved seed, or additional land. Most farmers do not have enough available income for large purchases such as these, and so they must make do without them. Because these farmers are cultivating pieces of land less than a hectare in area, or in the case of Uganda, 0.1 ha, it is very difficult to escape the downward cycle of barely scraping by without some type of additional resource, financial or otherwise (Sánchez, 2002).

A common response to the declining yields and increasing food insecurity observed in SSA is the introduction of aid in the form of food rations, inorganic fertilizer donations, and meal programs through non-government organizations (NGOs) or through government rationing programs (common in the 1980's especially). Instead of increasing food security and self-reliance, these NGOs focus more on the elimination of poverty and reduction of economic risk through aid programs (Devereux, 2016). A common issue observed in these type of situations is the inevitable reliance on "handouts" from these programs instead of reliance on one's own resources in a self-sufficient, sustainable way. Devereux (2016) summarizes Ethiopia's Productive Safety Net Program nicely, in that an important part of the program includes graduation to self-sufficiency, which is what the farmer works towards as they advance through the program. Increasing individual self-reliance within a single farm unit is important as

government resources become strained with increasing population pressures and decreasing national resources. It is necessary to understand the time and place for short term and long term aid when it comes to food security. The transition from short term aid should happen as soon as possible to prevent dependency and to equip farmers to rise up against adversity.

Recent Ugandan policy reflects a nationwide desire to improve the challenging situations many subsistence farmers find themselves in. A couple examples include the National Fertilizer Policy (2016) which aims to provide affordable and accessible mineral fertilizers to farmers, and the National Agricultural Extension Policy (2016) which was developed to address shortcomings of agricultural extension programs in rural communities and help subsistence farmers find more ways to develop commercial farm products which could be sold for additional revenue. There are many more laws in effect as well as being developed, but implementation on a national scale is a challenge regardless of intention. It is difficult to tailor national policy to each individual rural community for optimum success, necessitating local alternatives which are available to farmers until national changes can take effect.

As an alternative to mineral fertilizer application, the application of organic amendments is commonly recommended to farmers. As with mineral fertilizers, barriers preventing access to organic amendments exist in many rural areas. These barriers are primarily local in origin compared to the institutional issues that have contributed to the limited availability of inorganic fertilizers.

Sufficient soil carbon is an essential component of a healthy, functioning soil. Therefore, organic inputs are valuable for maintaining soil health for productive agricultural soils. They provide substantial benefit because they can enhance nutrient cycling, aggregation, soil water retention, and nutrient mineralization (Ashworth, 2017; Bastida, 2017; Ling, 2016; Sánchez, 2002), and they are especially vital because of their role in stimulating biological activity (Bastida, 2017; Ngosong, 2010; Plaza-Bonilla, 2016) and suppressing plant pathogens (Bonanomi, 2018). This is important because of the microbial role in biological N fixation, P solubilization, and other transformations (Nath, 2017; Sharma, 2013).

While organic amendments do supply soils with plant required nutrients and encourage soil health in other ways, these nutrients are often present in forms which are not immediately available to plants and require further breaking down by soil decomposers (Okalebo, 2007). Additionally, nutrient contents of these organic amendments can vary depending on soil nutrient contents in the areas where they are harvested. Biomass grown in nutrient poor soils may contain fewer plant nutrients than biomass grown in fertilized soils. In addition, animals feeding on nutrient-poor plant materials may also have lower levels of plant nutrients contained in their manures.

Despite this, Okalebo (2007) observed that in East Africa, maize yields on smallholder farms could be raised from below 0.5 t ha<sup>-1</sup> to 3-5 t ha<sup>-1</sup> through the use of organic inputs, generally manure and composts. These values were also observed at the study site from previous yield data (unpublished). Wokabi (1994) observed maize yields reaching a maximum without inputs at 1.5 t ha<sup>-1</sup>.

Organic fertility amendments such as composts or manures are a more accessible option than inorganic fertilizers, but an issue which is not discussed often is that access these amendments still requires a significant personal investment. Either a large volume of organic inputs is needed to supply enough nutrition to replace mined nutrients (Okalebo, 2007) which requires intensive labor, or there are personal barriers in place preventing people from accessing sufficient quantities of these inputs. Access to manure requires an investment in livestock or other small animals such as poultry or goats.

Acquisition of enough animals to produce a practical amount of manure is a luxury that many people cannot afford (Quiñones, 1997; Tittonell, 2013), and this varies by region based on primary land uses.

Variation of soil fertility and soil health within individual fields and family homesteads is often observed because of limitations when applying organic amendments. Whether the family has enough capital to purchase cattle is a high source of soil quality variability between farms and within different regions. When there are limited resources, only one part of the homestead repeatedly receives inputs due to location of the field in relation to the home (Tittonell, 2005; Tittonell, 2010; Zingore, 2007). It is often observed that any organic amendments such as small quantities of manures or kitchen scraps are applied to small home gardens instead of maize fields, which can sometimes be located a significant distance away. Without proper transportation tools, such as wheelbarrows or carts, transporting large quantities of manure is not a feasible task for many farmers. In addition, many animals (if present at all) are not kept in closed pens but are instead allowed to roam. Collection of manure

from free range chickens and goats is not practical and therefore not a suitable amendment when the home and family maize field is not located on the same plot of land. Lastly, any manure that the farmer manages to collect will often preferentially be used as cooking fuel in rural areas.

Given the above reasoning, if manure is not a suitable option, both uncomposted and composted plant biomass is the next best organic input. Composts are beneficial for their additions of soil carbon; however, they do contain fewer nutrients than manures. They can be created out of any plant material, including crop residues, brush, and cut grasses, all of which are available to farmers without financial cost. Most farmers do not have access to the proper equipment required for effectively incorporating residue back into the soil when it is un-composted, necessitating the creation of composts. For this practice to be successful, wide scale education is necessary to train farmers in proper composting techniques. The application of biomass, or mulch, in the form of cut grasses or crop residue poses a risk for attracting litter feeding termites, causing many farmers to remove all biomass and dispose of it or repurpose it off-site. Farmers also may burn all biomass as a method of removal, as observed with wheat farmers in Kenya (Okalebo, 2007).

Thousands of individual farms (0.1 ha) are often so degraded that they cannot produce enough biomass to compost residues on a practical scale, or cannot justify investing scarce resources into the production of green manure (Adamtey, 2016; Giller, 1997; Tittonell, 2013). The creation of compost requires labor in the form of turning piles and collection/transport of residues, which takes time and energy which some

farmers cannot justify expending. In addition, many farmers are not aware of composting techniques. Uganda is also diverse in crop production, leading to variable crop residue and biomass availability. Crops such as cassava, maize, tea, coffee, sweet potato, and beans all produce varying amounts of biomass (Braun, 1997), which also vary in nutrient density. This creates heterogeneity in types and amounts of biomass available for composing and green manure. In terms of fertility added, Woomer (1999) recorded nutrient content as a percent of dry matter of several types of organic inputs available in central Kenya. Sweet potato vines were found to add substantial N and K, more than cattle manure but not as much as poultry manure. Pigeon pea prunings, bean trash, and domestic compost also added comparable N & K. Maize stover added the highest amount of P, more than cattle or poultry manure. This could be a reflection of the feed available to the animals which would impact the quality of their manure. Local sweet potatoes are grown throughout Eastern Uganda, so this could be a decent source of organic fertilizer if farmers choose to incorporate it.

Another option could be the use of *Tithonia diversifolia* biomass as a compost or mulch for fields. *Tithonia* is a naturally occurring shrub located in the humid tropics of SSA which has been observed to accumulate nutrients in its tissue, and when used as an input in the absence of fertilizer it often doubled maize yields (Sánchez, 2002). The benefit here is that in addition to the N it is supplying in the place of a mineral fertilizer such as Urea, it is also providing trace amounts of micronutrients and K (Sánchez, 2002). As with manures discussed above, a drawback here is the labor involved with harvesting and transporting biomass from outside of the field to use as an amendment.

The other issue is that transporting plant biomass from one area to another disrupts natural nutrient recycling, as nutrients are being removed from one place and incorporated into another. This could decrease availability of brush resources in the future as plant material is consistently harvested from areas surrounding the farmland.

Another important consideration is also the variety of uses for crop biomass. In Uganda, crop biomass is often used as animal feed or bedding when applicable (Mtambanengwe, 2005) or sometimes as cooking fuel in the case of empty maize cobs (personal communication).

Other methodologies exist which can replace nutrients lost to cultivation. The two most common nutrient deficiencies in this region are N and P (Kihara, 2016; Olivera, 2004; Vance, 2001). Replacement of soil N is cheaply and successfully done by crop rotation with a legume (Vanlauwe, 2014), which has been observed by many local farmers in Eastern Uganda. Common beans (*Phaseolus vulgaris*) are a species of legume grown widely throughout this region and crop rotation is a concept being taught and adopted widely (Franke, 2018; Olivera, 2004; Plaza-Bonilla, 2016). This requires no financial investment as the N fixing bacteria are already present in the soil and Ugandan farmers are already growing common beans in their fields. The only change is to switch up the areas of the field where maize and beans are planted every couple growing seasons. Another approach is intercropping, where crops are grown throughout the same area instead of in separate sections. Intercropping of maize and common beans is a common approach in Kenya (Okalebo, 2007) but farmers are slow to adopt changes to generational farming practices.

The deficiency in soil P cannot be addressed through crop rotation. A common recommended practice to alleviate deficiency in available soil P is addition of organic amendments such as animal manure, however this is not a suitable option for many farmers. Uganda has substantial rock phosphate (230 x 10<sup>6</sup> tons) located the Sukulu Hill deposit (Okalebo, 2007), but it is low quality and unrefined, severely limiting any benefit. Some farmers have had marginal success in crushing it themselves for application but retrieving the rock phosphate and crushing it is labor intensive and many farmers live too far away from the deposits to benefit from them. Most often, deficiencies in P are addressed through small applications of whatever organic input is available.

Many farmers will adopt sustainable farming techniques for one to several growing seasons, only to revert back to traditional methods. Factors which have been proven to determine disadoption on rural farms include labor availability (organic amendments are costly to apply), credit constraints (difficult to purchase animals or fertilizer), risk and uncertainty in both the climate and with limited, valuable resources, and farm size (Marenya, 2007). In terms of this study, the labor is already being done (removal of mounds and disposal away from farm boundary). This labor will happen regardless of the recommendations in this study, meaning that excessive additional labor is not required as a part of using this amendment for many farmers. Because of this, farmers might be more likely to adopt any practices recommended in this study for the multiple growing seasons necessary to see improvements in their yields.

The purpose of this study is to evaluate a different, less studied amendment which is more widely available to subsistence farmers in many parts of Eastern Uganda. The effects of *Macrotermes* mound soil on maize yields have not been investigated thoroughly so a survey of mounds and a field trial were necessary to gauge the potential increase in grain and biomass production that this amendment could provide.

## 2.3. Termite Mound Soil as an Agricultural Input

One solution to the need to improve soil fertility throughout SSA could be the use of soil harvested from termite mounds. This resource is beneficial to subsistence farmers because it is a public organic resource, requiring no financial investment.

Farmers need only live near a mound to take advantage of the amendment. Many mounds are already being taken down and disposed of away from cultivated fields (Erens, 2015), so direct application to cropped soils is a better use of this resource. Many locals consider the mound-building termites pests, and a change in cultural perspective would need to occur in order to see widespread adoption of this practice, but it could prove invaluable to farmers who have limited access to other soil amendments.

There are many species of termites found around the world, but mound-building termites are concentrated mostly throughout Africa, Asia, and the Middle East (Oyedokun, 2009). Africa alone is home to around 1,000 individual species of termite. East Africa, a region encompassing Burundi, Ethiopia, Kenya, Madagascar, Rwanda, Somalia, Tanzania, and Uganda, is home to 177 species of termite (Kambhampati, 2000; Sileshi, 2010). These termites are typically grouped by food source: the litter feeders that

feed on leaf litter, dry grass stems, and some woody material, the soil feeders that feed on organic matter and humus attached to soil particles, and the wood feeders that feed exclusively on pieces of woody plant tissue (Paul, 2015). They can also be classified based on mound structure: termites can form above ground mounds (epigeal), below ground mounds (hypogeal), or no mound at all. This is further divided by whether they cultivate fungal colonies within mounds or not (Jones 1990).

This study focuses on the distribution, behavior, and agricultural impacts of *Macrotermes* mounds located in Eastern Uganda. Two main hypogeal, litter-feeding termite species, belonging to the *Terminitnae* subfamily *Macrotermitinae*, are common throughout this area: *Macrotermes bellicosus* and *Macrotermes subhualinus* (Pomeroy, 1978; Sileshi, 2010). *Pseudacanthotermes* is another common species of litter feeding termite in Uganda that build smaller mounds, and is generally regarded as less destructive than *Macrotermes bellicosus* (Pomeroy, 1976a). These termites can decompose a substantial quantity of litter at up to 80 % efficiency (Wood, 1978b). This plant material usually comes from a 50 m radius surrounding the mound (Wood, 1988), which when located near a cultivated field, can include crop residue. This is why termites are one of the primary decomposers of East African ecosystems, and also why many farmers consider them to be agricultural pests.

According to a survey of Ugandan mounds from 1976, there are 5 to 6 mounds per hectare in many parts of the country, and they grow at a rate of a cubic meter per year per mound, a rate that was similarly observed in Kenya (Keya, 1982; Pomeroy

1976a). Pomeroy (1977) reported that mounds reach a maximum density of about 8 mounds per hectare, predominantly in acacia woodland ecosystems (Wanyonyi, 1984).

Termites are an essential part of the East African Savanna ecosystem (Botinelli, 2015), sometimes creating their own ecosystem subtype known as a grouped tree savanna, where any trees present are associated with a termite mound. Total living termite biomass ranges from 70-110 kg ha<sup>-1</sup> in typical soils (Belyaeya, 2010), comprising between 40-65 % of total soil macrofaunal biomass (Wood, 1978c). Reports on number of termites per square meter varies widely, from 400 termites per square meter (Jones, 1990) to 15,000 termites per square meter (Wood, 1978a) in dry tropical soils.

High rates of nutrient turnover in these ecosystems can be attributed to termite activity (Kooyman, 1987), and their extensive influence on soils, vegetation, and biogeochemical cycling are also important ecosystem functions. Along with earthworms and some species of ants, termites are referred to as 'soil engineers' due to their extensive modification of ecosystems of the tropics and subtropics (Bottinelli, 2015; Jouquet, 2006; Paul, 2015).

Termite influence on biogeochemical cycling and soil modification is substantial (Jouquet, 2014), affecting soil carbon turnover, subsoil nutrient recycling, and nutrient mineralization. They are the dominant regulators of nutrient turnover in African Dry Woodlands (Jones, 1990) and other similar ecosystem types. Their extensive tunnel systems are essentially macropores (Zehe, 2010) which extend several meters surrounding and beneath the mound perimeter in a zone referred to as the

"termitosphere" (Jouquet, 2011; Lavelle, 1997). These macropores increase rainfall percolation (Bottinelli, 2015) especially in the structureless oxisols common in the region. These tunnels also open the soil up for increased gas exchange (Capowiez, 2006) and root exploration, overcoming the limitations of claypans and crusts common throughout SSA. In Burkina Faso, termite tunneling activity was manipulated in favor of farmers who fell victim to surface crusting in their fields. Zaï, traditional farming pits, were filled with cut grass to attract foraging tunnels of nearby termite mounds. As termites burrowed to retrieve the litter, tunnels were formed below and eventually through the crust to retrieve the litter and return it to their mounds. These tunnels and foraging holes are macropores which allowed for increased water infiltration and soil aeration, increasing the agricultural potential of the degraded fields (Kaiser, 2017).

Termite activity has also been observed to modify internal organization of soil aggregates (Bottinelli, 2015) and improve microaggregation through incorporation of soil carbon (Paul, 2015). In some parts of West Africa where poor soil structure is a constraint to agricultural production, termites have beneficial effects on soil structure that lead to significant crop improvement (Ouredraogo, 2006). However, Jouquet (2016) observed detrimental effects of termite activity on both macro- and micro-aggregate stability in the presence of water in an oxisol, a soil type common throughout East Africa, indicating that other variables most likely influence aggregation potential of termite activity.

An important role of termites in Uganda is their role in lowering the "stone line", a somewhat uniform layer of gravel which extends over most of the country. This layer,

historically, laid across the soil surface but through the subsoil redistribution of clay particles to the surface as mounds erode, termites have almost singlehandedly lowered the stone line to roughly 0.5-2 m below the surface (Pomeroy, 1976a), which is more favorable to agriculture. It has been estimated that the erosion of these mounds at a rate of 10 % per year, through natural weathering processes, deposits an average of 1.3 cm of topsoil per 100 years (Pomeroy 1976).

Mounds of fungus-growing termites will be described in more detail because of relevance to this study. The mounds themselves are built several meters tall out of subsurface clay particles (Abe, 2009; Millogo, 2011), typically a mixture of 1:1 layer silicates and other clay particles. *Macrotermes* mounds in Burkina Faso were observed to consist mostly of quartz, kaolinite, and K-feldspars (Millogo, 2011). An X-Ray diffraction (XRD) examination of Macrotermes mound soil in Western Tanzania revealed mainly metahalloysite and halloysite, at a ratio of 4:1 halloysite:smectite (Mahaney, 1999), which differs from the predominately kaolinitic mounds found elsewhere. The mounds in Tanzania were also found to contain concentrations of illite, quartz and orthoclase to varying degrees. Mounds located on an Oxisol in Southern India were found to contain a mixture of kaolinite, illite, talc, and smectite (Jouquet, 2016). Interestingly, Boyer (1948) observed that in some cases, the saliva of *Macrotermes* termites transforms illite clay to mica during the building process. These observations reflect the importance of underlying geology and soil pedologic processes on the physical composition of the mounds. The mineral composition influences mound chemistry and potential as fertility amendment. XRD data was not available for mounds

located in eastern Uganda, which may contain different minerals due to differences in underlying geology.

Because of their high clay content and rigid structure, mounds are more resistant to water infiltration and leaching (Sako, 2009). This prevents soluble silica from leaching from the mounds at the same rate as it is being leached from surrounding soil, leading to an enrichment in unstable clay minerals (Si:Al 2:1) contained in the mound (Ackerman, 2007; Mills, 2009). These minerals have a high affinity for cations, particularly trace elements when compared to the more stable, less negative minerals in the topsoil (Si:Al 1:1) (Nesbitt, 1997).

To construct these mounds, termites build extensive tunnel systems extending up to 10-12 m deep into the soil (Wood, 1988) to select wet, clay-sized soil particles, cementing them into tunnel and chamber walls (Paul, 2015). In the case of *Macrotermes* termites, they are unique in that they do not use fecal matter as a cementing agent in their mounds, instead relying on saliva as a binding agent for tunnels and gallery walls (Holt, 2000). These clay particles can be retrieved as deep as the water table (Holt and Lepage, 2000; Jouquet 2011) which is why some villages in Eastern Uganda use the termite mounds as indicators for groundwater when looking for suitable places to build wells (personal communication).

Fungus-growing termites have an established a mutualistic relationship with the basidiomycete *Termitomyces*, a specific species of fungus, which they "farm" within their mounds in specialized chambers (Um, 2013) by excreting and cultivating fungal spores housed in their gut biome. The fungus aids in the final step of the decomposition

process of foraged plant litter contained in termite feces (Visser, 2012), producing nodules which act as an easily digestible, N rich food source for the termites (Hyodo, 2000; Rohrmann, 1978; Um, 2013; Visser, 2012). This happens on top of specialized structures called fungus combs, pictured in figure 2.1. Though they are one of the top decomposers of lignin and cellulose in the region (Collins, 1981), termites will deposit this partially decomposed plant material along the top of the combs for the fungus to further decompose. These deposits serve as a continual food source for the fungus (Nobre, 2011), establishing mutual benefit.

The fungus-growing chambers, also referred to as galleries, are spread out throughout both above and below ground sections of the mound, and vary in size and shape, but are connected to each other and the outside of the mound through a series of tunnels. These galleries are temperature (25-30°C) and humidity (92 +/- 4 %) controlled in order to encourage *Termitomyces* growth within the mounds (Darlington, 1984; Jones, 1990; Singh 1981; Visser, 2012). This is accomplished through the systematic opening and closing tunnels with clay particles. This allows the galleries to remain at a constant temperature and moisture level year-round despite seasonal variability. Therefore, the fungus does not experience a fall in productivity that many surrounding plants experience during the bi-annual dry seasons of the region.



Figure 2.1 Examples of fungus galleries containing *Termitomyces* combs inside of two different *Macrotermes* mounds in Kyando, Uganda. Lighter colored, Nenriched nodules are visible on top of combs pictured on the right. Images photographed at the study site by Kimberlyn M. Pace.

These conditions are also favorable for pathogens, nematodes, and other antagonistic microbes (Blackwell, 1986; Rosengaus, 1998; Wood 1989; Zoberi, 1995). To overcome this, *Macrotermes* termites will preferentially select for *Termitomyces* using a variety of antifungal and antimicrobial mechanisms.

According to previous chemical analyses of the mound soil, plant essential soil nutrients are commonly concentrated within the lower central zone of most mounds (Erens, 2015), specifically N, P, Potassium (K), Calcium, Magnesium, and Sulfur (S) (Erens, 2015; Hesse, 1955; Pomeroy, 1976; Sarcinelli, 2009; Sileshi, 2010; Watson, 1977). Of particular interest is available P, where high concentrations have been

observed within mounds compared to surrounding topsoil (Rückamp, 2009). A chemical analysis of *Macrotermes* mounds in 1977, using the anion exchange resin method, reported that the mounds contained 37 mg kg<sup>-1</sup> of available P in the mounds, compared with 10 mg kg<sup>-1</sup> in the surrounding topsoil Ah horizon and 5 mg kg<sup>-1</sup> in the Ap horizon (Watson, 1977), and a 2010 study of *Macrotermes* mounds in Kenya reported that there was 20% more P in mounds than in surrounding topsoil, although the results were not significant (Sileshi, 2010). However, soil P values tend to vary substantially between studies, indicating a strong influence of sample location and the inherent mineralogy of the soils from which the mounds are derived (Jouquet 2011). A chemical analysis of mound soil in Rhodesia concluded that in terms of crop benefit, mixing mound soil with field topsoil will primarily add calcium and magnesium to the soil (Watson, 1977).

A micronutrient study of mounds in Namibia (Sako, 2009) reported that higher concentrations of Iron, Manganese, Cobalt, and Nickel were present in the mounds, although variation was observed between sites. Less is known about the distribution and accumulation of micronutrients compared to macronutrients in *Macrotermes* mounds (Ackerman, 2007; Mills, 2009). Mounds consisting primarily of halloysite in Western Tanzania were proven to contain elevated levels of aluminum compared to surrounding topsoil (Mahaney, 1999).

Concentrations of plant nutrients contained in *Macrotermes* mounds were found to vary significantly with underlying geology. In a study of southern African mounds, large mounds located on low fertility granite were found to significantly concentrate

plant available nutrients into 'fertile islands', while those located on nutrient rich basalt were not enriched (Muvengwi, 2017).

It can be inferred that because mounds are moderately more fertile than surrounding topsoil, these nutrients are stored within the mound until they are eroded or anthropogenically destroyed. It is important to note that the fertility sequestered and stored within the mounds is dependent on location, soil type, local land use, climate, and many other factors which influence the accumulation and distribution of plant essential nutrients and organic matter.

*Macrotermes* mounds may have a higher pH than surrounding topsoil (Erens, 2015; Sarcinelli, 2009; Watson, 1977), and varying amounts of decaying organic matter due to their saliva, excretory material, and the mutualistically related fungus combs (Duponnois, 2005; Holt, 1998; Pomeroy, 1976a). The quality of this organic matter is debatable. Harris (1949) observed that the organic matter contained within the mounds is digested to the point where it provides only slight organic enrichment to the surrounding soil.

These mounds can be considered 'hubs' of biological activity just as they are thought of as 'islands' of vegetation in many savanna ecosystems. Meiklejohn (1965), Keya (1982), and Fox-Dobbs (2010) each found increased concentrations of nitrifying bacteria within the mounds. This could also indicate a greater concentration of plant accessible N. Additional soil biological activity can also be observed within select mounds. Secondary termites, several different species collectively referred to as "inquilines" (Rückamp, 2009), commonly occupy mounds that have been abandoned by *Macrotermes* termites. These non-mound building termites will inhabit the structures

built by *Macrotermes* without upholding the integrity of the structure while at the same time changing the chemistry and fertility status of the mound (Rückamp, 2009).

Some of the less beneficial actions of these termites are what encourage many farmers to remove the mounds from their property. Litter feeding termites take plant residues, which would otherwise decompose in the field, to a centralized location away from the field where they are decomposed. Little is left to return organic matter and nutrients to the soil (Jones, 1990), and farmers remove the mounds to prevent loss of crop residue, disposing of them even further away. Termites will redistribute soil carbon down into the subsoil through fecal matter, saliva, and the decomposition of their dead, which in turn may improve soil structure and microaggregation in the soil around the mound (Bottinelli, 2015). However, the benefits to the soil in the area under the mound do not compensate for the much greater loss of carbon in a several-meter radius around the mound in the form of biomass removal.

A study of mounds in Western Tanzania actually found less organic carbon in the mounds compared to surrounding topsoil (Mahaney, 1999). Generally, litter feeding termites concentrate organic carbon within their mounds, while *Macrotermes* termites tend to have lower concentrations of organic carbon compared to surrounding topsoil (Holt, 2000). All of the plant essential nutrients mineralized during decomposition are also stored indefinitely until the mound is taken down or eroded (Gosling, 2012; Jones 1990). This erosion process could take anywhere from months to decades depending on climactic factors as well as the integrity of the mound structure and overall mineral composition (Jouquet, 2011). For example, Lepage (1984) estimated complete erosion to

occur in 20-25 years for a typical *Macrotermes bellicosus* mound (around 8 m<sup>3</sup> in volume).

Additionally, litter-feeding termites have been known to feed on crop residues and even parts of the living crops themselves. Pseudacanthotermes termites feed on live maize roots by covering them with a sheeting of soil particles and slowly bringing the tissue back to their nests (Paul, 2015). Any mulches, either cut grass or crop residue, are also vulnerable to termite attack. If maize stalks are blown over by wind, or otherwise lodged, parts of the cob, stalks, or roots could potentially be removed by the actions of litter feeding termites. This can be a problem in Uganda, where field drying of mature grain on the stalk for up to 4-7 weeks is the most common way to prepare maize for processing and storage (Kaaya, 2005). Many local farmers near the study site are hesitant to apply mulch or any other soil cover to their fields that might attract termites (personal communication with area farmers; Paul, 2015; Tian, 1993). This was investigated with a study from 2014, which examined the volume of maize surface residue lost to termite feeding with and without measures taken to remove termites from the site (pesticides). No significant differences were observed in macrofauna levels in the mulched plot versus the non-mulched plot, indicating that the addition of mulch did not attract increased populations of termites to the field. They found that instead, tillage had more of a controlling effect, where tilling actively prevents termites from building their tunnel systems through the field (Kihara, 2014). However, the same study observed that with the exclusion of the macrofauna altogether, 20 % of surface residue was lost over the growing season compared to 85 % without the exclusion. Other studies in drier

climates found that maize grown in fields with macrofauna outperformed maize where they were removed (Dawes, 2010; Evans, 2011). Kooyman and Onck (1987) also observed that tillage forced termites to vacate cultivated fields, abandoning construction of tunnels inside regularly plowed soil. This phenomenon prevents many farmers from adopting no-till or reduced-till farming (personal communication with area farmers).

An interesting observation from this study is that while termites will hollow out the center of the cob for food, they will leave the grain almost entirely untouched and able to be consumed by people. However, it is not protected from other foraging animals such as rats and moles that can access the grain. If termite damage to crops is early enough in the season, crop growth will be stunted and grain yields decreased. Tunnel systems, if extended into the field, can weaken the integrity of the soil and cause problems for rooting stability in maize plants, which is detrimental at both the development stages and the drying stages. If galleries and chambers are present near the soil surface, they can pose a physical hazard to the farmer walking across the field. Mounds are also very large and difficult to remove, causing more work for farmers who already work hard to tend to their land.

Research interests in the usefulness of soil from litter-feeding termite mounds has not been as substantial as that of soil feeding termites. Prior research focused on their role as agricultural pests or their benefit to ecosystem services rather than their potential benefit to agricultural systems (Paul, 2015). Therefore, one goal is to renew interest in alternative amendments present around the study site as well as address some of the

questions that remain unanswered regarding the potential benefit that termite mounds can provide to the degraded soils of rural eastern Uganda.

The usefulness of *Macrotermes* mound soil as a fertility amendment is highly dependent on location, with the chemistry varying significantly even within the same landscape when it comes to position on a slope (Pomeroy, 1977). A variety of studies were published on the chemical composition of *Macrotermes* mounds in many different countries, all of which differ from each other, indicating that local applicability of this amendment as a source of fertility would require regional testing before a recommendation could be made.

Because the fertility and organic material contained in these mounds is tied up until they are eroded or knocked over by man, their usefulness in natural enrichment is debatable. Pomeroy (1976b) estimated that around 10 % of the mound volume is anthropogenically destroyed per year in Uganda, paired with around 10 % lost to natural erosion. These losses are counter balanced by an annual growth rate of around 1 m<sup>3</sup> per mound.

Watson (1977), after studying *Macrotermes falciger* mounds, concluded that the mounds were a beneficial amendment to cultivated soils through incorporation of the mound soil into the topsoil of the field. He observed increased concentrations of N, P, Calcium, and Magnesium in these mounds. Similarly, he also observed increased concentrations of Calcium, Magnesium, and available N in *Macrotermes* mounds from Rhodesia when compared to surrounding topsoil. Pomeroy (1976a) recorded increased concentrations of N, P, Calcium, and K in Ugandan *Macrotermes* mounds. Hesse (1955)

studied mounds located in the drier regions, where he found that increased calcium accumulations in the mounds led to the formation of nodules. Meiklejohn (1955) observed increased nitrifying bacteria, cellulose decomposers, and denitrifiers in mound soils compared to surrounding topsoil. This concept of stimulated microbial activity is mirrored in a more recent study done in Kenya where increased numbers of fungi, bacteria, and actinomycetes were recorded in dead *Macrotermes* mounds than living (Keya, 1982).

A study of mounds in the DRC contradicted the assumption of higher fertility compared to surrounding topsoil because soil taken from older mounds was found to be infertile and formed sterile patches where they were eroded (Meyer, 1960). Nye (1955) observed that *Macrotermes bellicosus* mounds in Nigeria were less fertile than the topsoil surrounding the mounds, and that grain yields decreased when maize was grown on collapsed mounds compared to surrounding soil.

Even if termite mounds are proven to be moderately more fertile than surrounding topsoil, their impact on crop yields is debatable. Pomeroy (1976a), despite finding increased concentrations of several plant essential nutrients, concluded that it was not enough to benefit sustenance farmers in Uganda when used as the sole fertility amendment. In fact, he stated that the most important role of termites in this region was not to increase fertility of the soil, but instead to continue lowering the stone line through the creation of a stone free topsoil layer as they are eroded.

Despite this, a handful of local farmers near the study site have observed maize yield increases when discarding mound soil on their fields instead of removing the

mound altogether (personal communication), prompting this study. An additional personal observation is that vegetation growing on top of the mounds was noticeably greener than surrounding vegetation, indicating a potential increase in N.

## 2.4. Arbuscular Mycorrhizal Fungi

Arbuscular Mycorrhizal Fungi (AMF) are essential to the productivity and functioning of agricultural systems due to their ability to sequester plant nutrition (P) and water. Therefore, mycorrhizal fungi infection density was used as an indicator to gauge the robustness of the microbial community present as influenced by amendment application.

Arbuscular mycorrhizal fungi are found throughout most of the world (Baum, 2015), in most soil types and conditions (Hetrick 1984; Mosse, 1981) except for arid soils and soils which are heavily degraded. In such soils, they are found in much smaller concentrations if at all (Alguacil, 2009). Up to 80 % of land species will form these associations (Baum, 2015), including most agricultural crops. Despite the degraded state of many soils found in East Africa, AMF are still found throughout the region and interactions between AMF and many crops have been recorded.

AMF are classified in the phylum *Glomermycota*, which includes the fungi that form a symbiotic relationship with a variety of host plants (Alguacil, 2009). There are three families of AMF species, the *Acaulosporaceae*, *Gigasporaceae*, and *Glomaceae* (Dodd, 2000; Morton, 1990; Morton, 1994). Examples of host plants include readily infected field crops such as maize, sorghum, onions, and clover, and hundreds of

additional species. Other plants do not respond as favorably to AMF colonization, including wheat, rice, and barley, but have still been observed to form associations in special circumstances. The degree of fungal infection varies based on cultivar as well. For example, AMF colonized different cultivars of wheat to varying degrees when all other conditions were kept constant (Hayman, 1982). Hosts excluded from this association are plants from the families *Brassicae* (Hayman, 1982) and *Chenopodiaceae* (Newman, 1987) which have been observed to excrete antagonistic compounds preventing AMF colonization (Baum, 2015; Hayman, 1982). These compounds can remain in the soil following the removal of the antagonistic plant, stunting future mycorrhizal proliferation (Dalpe, 2004; Smith, 1997).

These fungi are classified as obligate biotrophs (Hayman, 1982), meaning their only source of carbon is acquired from the host plant instead of the decomposition of organic materials (Hodge, 2001). This is a predominantly symbiotic relationship, in that both host and fungi benefit, but there are circumstances where the fungi can become parasitic, decreasing the host plant's ability to function (Lerat, 2003). This usually occurs in soils with high available P, where forming a symbiosis for P excavation is no longer a competitive advantage over other non-mycorrhizal plants (Hayman, 1982). In this case, the plant will sometimes employ anti-fungal agents to decrease the fungi's ability to penetrate and colonize the root (Iqbal, 1976). Due to the low P levels observed throughout East Africa, this is not commonly observed and AMF associations are generally regarded as beneficial because they aid in the acquisition of elusive soil P outside of the root depletion zone.

A defining quality of AMF is the ability to form arbuscules in the cortical cells of host plants. Arbuscules are branching structures which increase fungi-plant interaction area for nutrient and photosynthate exchange (Bever, 2011). They are rectangular in shape because they fill the space of the cortical cell while remaining within a cell wall boundary layer, never truly penetrating the cell. An example is shown in figure 2.2 (C). This transfer of P from fungi to host plant through the arbuscules is thought to be the primary driver for plants to form these symbioses (Graham, 2000). Some species of AMF will form vesicles in addition to arbuscules, which are swollen hyphae that act as storage structures for plant-provided carbon (Biermann, 1983; Mosse, 1973; Staddon, 1998). These can occur both internally and externally depending on the species, an example is shown in figure 2.2 (B, C, D). The formation of these structures indicates a highly successful, mature association, compared to the presence of fungal hyphae only. The presence of hyphae simply means the fungi and plant have agreed to work together, while increased numbers of arbuscules provides evidence of higher rates of transfer between the plant and fungi, and increased vesicles indicates greater volume of transfer.

In return for supplying host plants with soil nutrients and water, AMF receive up to 20 % of carbon produced in the host plant through photosynthesis (Gosling, 2006; Jakobsen 1990; Parniske, 2008). Because they are unable to assimilate any other sources of carbon, this is a key benefit for the fungi. This can sometimes be thought of as an investment from the plant into the soil carbon pool, which stimulates other microbial activity and nutrient breakdown from organic carbon sources.

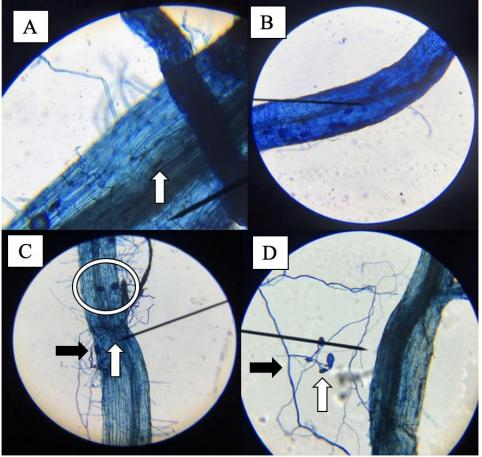


Figure 2.2 Mycorrhizal structures observed colonizing maize roots (*Zea mays*) in Kyando, Uganda. Roots were cleared with potassium hydroxide and photographed by Kimberlyn M. Pace under 10x magnification using an Iphone 7 camera. (A) An arbuscule, (B) a cluster of vesicles, (C) two vesicles (white circle), an arbuscule (black arrow), hyphae (white arrow), and (D) extracellular hyphae (black arrow) and an external vesicle (white arrow).

Soils are heterogeneous and highly diverse, differing by texture, microclimate, types of vegetation, and water availability. Because of this, they often contain many different species of AMF (Abbott, 1991). The variation in pH (Abbott, 1985), soil P status (Abbott, 1984; Schwab, 1983) and soil type (Abbott, 1985) will also be key determinants of which species of AMF are present. For example, the *Glomus* species has

difficulty establishing successful infections in low pH soils (Abbott, 1985). These diverse soil conditions contribute greatly to the diversity of fungal species able to colonize viable hosts.

Land use also has an effect on species diversity in that monoculture agroecosystems, over time, will preferentially select for specific species which benefit them, causing other species to move elsewhere or decline in population density (Oehl, 2003). Rotation with a non-mycorrhizal host crop can also have a detrimental effect on AMF proliferation, even extending into future growing seasons when the AMF-viable crop is returned to the field (Douds, 1997; Gavito, 1998).

Numerous species can also colonize a specific root segment at once (Tommerup, 1988). This is where identification issues can arise; there is a lack of information published about differentiating species from one another, so many AM species remain uncharacterized (Abbott, 1991). In addition, observing specific diagnostic structures can be difficult especially when examining external mycelium from field samples (Abbott, 1985).

Given these diagnostic limitations, there are some defined clues which can be used to gain a better understanding of the different types of AM fungi which may be present on the roots in question. Around 120 species of AMF have been successfully classified by visual means (Morton, 1988). Many of these were classified using single-species pot studies (Merryweather, 1998), which are not necessarily reflective of field conditions, but can still give helpful insight. Despite this, field identification at the

species level remains difficult, necessitating the use of more general classification systems (Merryweather, 1998).

Mycorrhizal fungi are physiologically differentiated from each other in several ways. Different species of AM fungi will display differences in hyphal branching patterns, hyphal infection density, and hyphal thickness (Abbott, 1985). Infection generally ranges between 48-84 % depending on species observed in the field (Sutton, 1973). Furthermore, some species do not form external hyphae, and others do (Graham, 1982). Of those that do, the proportion of external hyphae produced per segment of root length differs (Abbot, 1985) as well as types and length of strands (Brundrett, 1996).

For example, *Glomus fasciculatum* produces less external hyphae per centimeter of infected root than *Gigaspora calospora*, but when infection density was examined per centimeter root length, *Gigaspora calospora* had a lower average infection density (Abbott, 1985). *Glomus* species generally have straight hyphae with 'H' shaped branches (Dodd, 2000). These are typically found in maize, sorghum, leek, and onion (Dodd, 2000) with the exception being *Glomus occultum*, which is not (Morton, 1985). *Acaulospora* are differentiated from *Glomus* in that their hyphae are less straight and more irregular, still exhibiting 'H' shaped branches. Their hyphae are thinner and looped when compared to *Glomus*, and vesicles are irregularly shaped (Dodd, 2000). Species belonging to *Gigaspora* will grow wider diameter, looping hyphae and the trucks of their arbuscules will be thicker as well (Morton, 1995). Differences in hyphal thickness are displayed in figure 2.3.

Something to note is that when it comes to hyphal length, it seems to be more dependent on soil nutrient status and distribution rather than the occurrence of specific mycorrhizal species (Abbott 1985). AMF may also be classified based on glomalean spore type (Bentivenga, 1992; Morton, 1990). This method is unreliable because these types of spores are produced on external mycelium, so when one is found it is sometimes difficult to determine the fungi which produced it (Merryweather 1998).

Furthermore, fungi may be differentiated from each other by examining their vesicles. *Glomineae*, a suborder of AMF fungi, will form vesicles in the cortical cells of the host plant. Those which have been observed to form intraradical vesicles include *Glomus fasciculatum*, *Glomus mossease*, *Acaulospora spinose*. Those which do not form these types of vesicles include *Gigaspora margarita* and *Gigaspora gigantea* (Biermann, 1983). *Gigaspora margarita* has also been observed to form extraradical vesicles, however these were not successful when used as inoculum whereas the intraradical vesicles from species listed above were (Biermann, 1983). Vesicles of *Glomus* fungi are generally oval shaped, sometimes elliptical, and sometimes will form a thickened, spore producing wall (Dodd, 2000). *Gigaspora* form auxiliary cells at ends of external hyphae instead of vesicles (Dodd, 2000).

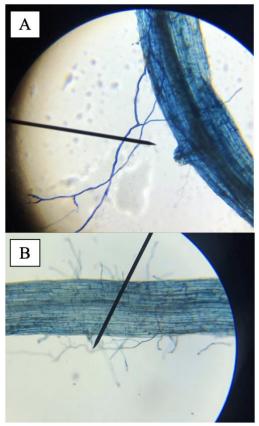


Figure 2.3 Differences in hyphal thickness observed between two maize (*Zea mays*) root samples in Kyando, Uganda. Roots were cleared with potassium hydroxide and photographed by Kimberlyn M. Pace under 10x magnification.

## 2.4.1. Importance to Smallholder Agriculture

AMF can be thought of as an indirect source of fertilizer for smallholder farmers cultivating degraded soils, among other benefits related to water acquisition in drought conditions. Extraradical hyphae act as extensions of root surface area, extending past the nutrient depletion zones of fine roots to scavenge for immobile nutrients such as P and zinc (Joner, 1995; Muleta, 2008; Sanders, 1971; Thomson 1987; Toljander, 2006). This potentially increases plant productivity (Koide, 2000; Sylvia, 1988; Yang, 2016) in low

input farming systems (Jakobsen, 2005). It has also been proposed that mycorrhizal roots could secrete higher concentrations of phosphatases into the soil, which work to free up P that has been tied up in unavailable forms in the soil (Jayachandran, 1989; Jayachandran, 1992; Mitchell, 1981; St. John, 1983; Warner, 1984). Increased levels of micronutrients have also been observed to be taken up by the extraradical hyphae of AMF (Azaizeh, 1995). Smallholder agriculture is characterized by an overall lack of inputs, especially mineral fertilizers. AMF are already present in most soils, and as long as no inoculant is purchased, their services are completely free to farmers who could otherwise not afford fertilizers or other agricultural products.

AMF also provide numerous benefits to soil structure through deposition and movement of plant assimilates (Andrade, 1997; Johnson, 2002) and glomalin, an extracellular glycoprotein which sticks mycelium to soil (Gosling, 2006). As these compounds are deposited and particles stick to the hyphae, soil microaggregation is increased in the surrounding soil, improving soil stability (Degens, 1996; Ravenskov, 1999). There is uncertainty in how much of a role that glomalin plays in soil aggregation (Franzluebbers, 2000). Host plants benefit from increased disease and pathogen resistance (Borowicz, 2001; Gance, 1994; Pozo 2002; Smith, 2008).

Lastly, AMF has been observed to improve plant response to mild water stress (Augé, 2004; Baum, 2015; Davies, 2002) by improving root hydraulics and giving the plant the ability to generate enough matric potential to take up water in smaller, more tightly held pores. This is extremely important in rain-fed agricultural systems, where

irrigation is not widely available and water is a limited resource. This also may increase access to water soluble soil nutrients which are dissolved in soil solution.

Farming practices which increase AMF proliferation and hyphal branching in soil include the application of organic matter (Jayachandran, 1992; St. John, 1983), reduction of tillage, and crop rotation with other mycorrhizal plants (Oehl, 2003). In low-input systems where there is limited or non-existent fertilizer application, farmers need to rely on alternative methods such as AMF to the accessibility of plant nutrients in their fields.

## 2.5. Interactions between Termite Mound Soil and AMF

Macrotermes termites preferentially select for Termitomyces fungus, though the exact mechanism by which this is done is unclear. There are many proposed methods by which litter feeding termites control microbial and fungal populations in their mounds. One is by secreting an antimicrobial substance known as bacillaene A(1), a metabolic by-product produced from a strain of Bacillus in the termite gut (Rosengaus, 1998); this compound prevents colonization of other species of fungus besides Termitomyces (Um, 2013). This antimicrobial compound is found in the fecal matter of these termites, who then could potentially use it as part of the mound structure (Rosengaus, 1998). That way, the antimicrobial compound is distributed throughout the mound to control fungal movement and distribution. The antifungal properties of this fecal matter are very active and the suppressive effects can be noticed almost immediately (Rosengaus, 1998) in that

they limit the germination of fungal spores and subsequent growth of any fungi outside of *Termitomyces* (Wood, 1989).

Another proposed origin of the anti-microbial substance found in termite fecal matter is from digested plant litter. Litter-feeding termites are digesting a variety of plants, some of which may naturally contain antibacterial substances and tannins (Sikorowski, 1994). Instead of being fully broken down, these compounds could pass through the gut of the termites and be deposited as fecal matter throughout the nests. In addition to this, Ayitso (2015) proposed two other types of antibiotics that could be the source of anti-fungal properties of termite fecal matter. These antibiotics are termicin and spinigerin, which also protect the *Termitomyces* fungus from antagonistic fungi and microbes. Visser (2012) proposed that because actinobacteria were observed throughout the mounds, they could be contributing defensively to the antimicrobial properties found in the mound.

These proposed methods of mound building termites controlling fungi within the mound directly conflict with the observation that *Macrotermes* termites do not use fecal matter as a structural component (Jouquet, 2011). Therefore, the fecal matter which is deposited on fungal combs could potentially be the source of the antimicrobial presence rather than tunnel and gallery walls.

In conclusion both AMF and termite mound soil have the potential to benefit subsistence farmers who otherwise cannot apply soil amendments. For farmers in eastern Uganda, the benefit was quantified by this study in an attempt to gauge the effectiveness and applicability of these two resources.

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#### 3.1. Introduction

There are many species of termites found around the world, but mound-building termites are concentrated mostly throughout Africa, Asia, and the Middle East (Oyedokun, 2009). Africa alone is home to around 1,000 individual species of termite. East Africa, a region encompassing Burundi, Ethiopia, Kenya, Madagascar, Rwanda, Somalia, Tanzania, and Uganda, is home to 177 species of termite (Kambhampati, 2000; Sileshi, 2010). These termites are typically grouped by food source: the litter feeders that feed on leaf litter, dry grass stems, and some woody material, the soil feeders that feed on organic matter and humus attached to soil particles, and the wood feeders that feed exclusively on pieces of woody plant tissue (Paul, 2015). They can also be classified based on mound structure: termites can form above ground mounds (epigeal), below ground mounds (hypogeal), or no mound at all. This is further divided by whether they cultivate fungal colonies within mounds or not (Jones, 1990).

This study focuses on the distribution, behavior, and agricultural impacts of *Macrotermes* mounds located in Eastern Uganda. Two main hypogeal, litter-feeding termite species, belonging to the *Terminitnae* subfamily *Macrotermitinae*, are common throughout this area: *Macrotermes bellicosus* and *Macrotermes subhualinus* (Pomeroy, 1978; Sileshi, 2010). According to a survey of Ugandan mounds from 1976, there are 5 to 6 mounds per hectare in many parts of the country, and they grow at a rate of a cubic meter per year per mound, a rate that was similarly observed in Kenya (Keya, 1982;

Pomeroy 1976a). Pomeroy (1977) reported that mounds reach a maximum density of about 8 mounds per hectare, predominantly in acacia woodland ecosystems (Wanyonyi, 1984).

Termite influence on biogeochemical cycling and soil modification is substantial (Jouquet, 2014), affecting soil carbon turnover, subsoil nutrient recycling, and nutrient mineralization. They are the dominant regulators of nutrient turnover in African Dry Woodlands (Jones, 1990) and other similar ecosystem types. Their extensive tunnel systems are essentially macropores (Zehe, 2010) which extend several meters surrounding and beneath the mound perimeter in a zone referred to as the "termitosphere" (Jouquet, 2011; Lavelle, 1997). Perhaps the most important role of termites in Uganda is their role in lowering the "stone line", a somewhat uniform layer of gravel which extends over most of the country. This layer, historically, laid across the soil surface but through the subsoil redistribution of clay particles to the surface as mounds eroded, termites have almost singlehandedly lowered the stone line to roughly 0.5-2 m below the surface (Pomeroy, 1976a), which is more favorable to agriculture. It has been estimated that the erosion of these mounds at a rate of 10 % per year, through natural weathering processes, deposits roughly 1.3 cm of topsoil per 100 years (Pomeroy, 1976a).

The mounds themselves are built several meters tall out of clay particles (Abe, 2009; Millogo, 2011). Termites build extensive tunnel systems extending 10-12 m deep into the soil (Wood, 1988) to select wet, clay-sized soil, cementing the particles into tunnel and chamber walls (Paul, 2015). A majority of particles are selected from the top

meter of the soil profile however (Pomeroy, 1976a). In the case of *Macrotermes* termites, they are unique in that they do not use fecal matter as a cementing agent in their mounds, instead relying on saliva as a binding agent for tunnels and gallery walls (Holt, 2000). Fungus-growing termites have an established a mutualistic relationship with the basidiomycete *Termitomyces*, a specific species of fungus, which they "farm" within their mounds in specialized chambers (Um, 2013) by excreting and cultivating fungal spores housed in their gut biome. The fungus aids in the final step of the decomposition process of foraged plant litter contained in termite feces (Visser, 2012), producing nodules which act as an easily digestible, N rich food source for the termites (Hyodo, 2000; Rohrmann, 1978; Um, 2013; Visser, 2012).

According to previous chemical analyses of the mound soil, plant essential soil nutrients are commonly concentrated within the lower central zone of most mounds (Erens, 2015), specifically N, Phosphorus (P), Potassium (K), Calcium, Magnesium, and S (Erens, 2015; Hesse, 1955; Pomeroy, 1976; Sarcinelli, 2009; Sileshi, 2010; Watson, 1977). Of particular interest is P, where high concentrations have been observed within mounds compared to surrounding topsoil (Rückamp, 2009). A chemical analysis of *Macrotermes* mounds in 1977, using the anion exchange resin method, reported that the mounds contained 37 mg kg<sup>-1</sup> of available P in the mounds, compared with 10 mg kg<sup>-1</sup> in the surrounding topsoil Ah horizon and 5 mg kg<sup>-1</sup> in the Ap horizon (Watson, 1977), and a 2010 study of *Macrotermes* mounds in Kenya reported that there was 20% more P in mounds than in surrounding topsoil, although the results were not significant (Sileshi, 2010). A micronutrient study of mounds in Namibia (Sako, 2009) reported that higher

concentrations of Iron, Molybdenum, Cobalt, and Nickel were present in the mounds, although variation was observed between sites. Less is known about the distribution and accumulation of micronutrients compared to macronutrients in *Macrotermes* mounds (Ackerman, 2007; Mills, 2009).

It can be inferred that because mounds are moderately more fertile than surrounding topsoil, these nutrients are stored within the mound until they are eroded or anthropogenically destroyed. It is important to note that the fertility sequestered and stored within the mounds is dependent on location, soil type, local land use, climate, and many other factors which influence the accumulation and distribution of plant essential nutrients and organic matter.

One solution to the need to improve soil fertility throughout SSA could be the use of soil harvested from termite mounds. This resource is beneficial to subsistence farmers because it is a public organic resource, requiring no financial investment. Farmers need only live near a mound to take advantage of the amendment. Many mounds are already being taken down and disposed of away from cultivated fields (Erens, 2015), so direct application to cropped soils is a better use of this resource.

Some of the less beneficial actions of these termites are what encourage many farmers to remove the mounds from their property. Litter feeding termites take plant residues, which would otherwise decompose in the field, to a centralized location where they are thoroughly decomposed. Little is left to return organic matter and nutrients to the soil (Jones, 1990), and farmers remove the mounds to prevent loss of crop residue. Soil carbon is stored in the mounds and removed from the soil carbon cycle until the

mound is knocked down. Litter feeding termites redistribute soil carbon down into the subsoil through fecal matter, saliva, and the decomposition of their dead, which in turn improves soil structure and microaggregation in the soil around the mound (Bottinelli, 2015). The benefits to the soil in the area under the mound do not compensate for the much greater loss of carbon in a several-meter radius around the mound. Many local farmers near the study site are also hesitant to apply mulch or any other soil cover to their fields that might attract termites (personal communication with area farmers; Paul, 2015; Tian, 1993).

Research interests in the usefulness of soil from litter-feeding termite mounds has not been as substantial as that of soil feeding termites. Prior research focused on their role as agricultural pests or their benefit to ecosystem services rather than their potential benefit to agricultural systems (Paul, 2015). Therefore, one goal is to renew interest in alternative amendments present around the study site as well as address some of the questions that remain unanswered regarding the potential benefit that termite mounds can provide to the degraded soils of rural eastern Uganda. The usefulness of *Macrotermes* mound soil as a fertility amendment is highly dependent on location, with the chemistry varying significantly even within the same landscape when it comes to position on a slope (Pomeroy, 1976a). A variety of studies were published on the chemical composition of *Macrotermes* mounds in many different countries, all of which differ from each other, indicating that local applicability of this amendment as a source of fertility would require regional testing before a recommendation could be made. A survey was conducted which included 34 mounds located in rural Eastern Uganda to

evaluate the usefulness of this resource as an amendment for subsistence farmers located near the study site.

### 3.2. Methods

# **3.2.1. Study Site**

The study site is located on a 8.9 hectare teaching farm owned by the non-profit Vocare Ministries Ltd., located in Kyando, Mayuge District, Uganda. Located in the Lake Victoria Crescent at 00°25.826N and 033°26.013E, the farm property is on a gentle slope towards a small valley. Thirty-four mounds were mapped and analyzed for the survey, displayed in figure 3.1. The density of mounds observed on this property is less than what was recorded in prior surveys of *Macrotermes* mounds in Uganda. This could be due to removal of mounds by farmers, or due to soil type. The soil texture of this study site is a sandy loam, the presence of sand is likely a result of proximity to Lake Victoria, located 7 km to the southwest. Mounds are less likely to form in high densities in soils with low clay content. The soil order at the site is a Nitisol (Bakamanume, 2010). Nitisols are similar to the USDA soil orders of Alfisols or Ultisols, and common throughout the tropical highlands of Kenya, Ethiopia, and the Democratic Republic of the Congo (DRC). They are fine textured and intermediately weathered, with clay contents around 30 %. They are productive for agriculture if managed properly.

The ecosystem type is grouped tree savanna, with a bi-annual rainy season. On average, it rains on this site 160-170 days per year, with rainfall of moderate intensity at

1500-1750 mm annually (Bakamanume, 2010). The average temperature is 24°C during the dry season and 22°C during the rainy season.

Elevation is approximately 3800 ft above sea level (World Bank, 2016) as the site is positioned on a large plateau which encompasses most of Uganda. Soils in this area are likely to have been weathered from the granites, quartz, and acid gneisses of the Basement Complex, in addition to alluvial or lacustrine sediments (Bakamanume, 2010).

## **3.2.2. Physical Survey**

A survey of 34 mounds included estimated height, volume, percent and type of vegetative cover, state of erosion, presence of living and secondary termites, and landscape position. Mound locations are displayed in figure 3.1. Species of mound building termites in this region tend to build in concentrated clusters of the same species instead of interspersed (Pomeroy, 1978), with the result that a majority of the mounds are inhabited by *Macrotermes* termites. Mound height was calculated using reference photos with a ruler included for scale. Total above ground volume of each mound was estimated by using height measurement, hypotenuse length, and base widths with the assumption that the mounds are roughly cone shaped.



Figure 3.1 Map displaying the location of all *Macrotermes* mounds located at the 22-acre Vocare Ministries Farm in Kyando, Uganda. The map was created using Google Maps and GPS coordinates from a handheld Garmin GPS.

Ranking systems were used to characterize the observational data collected for mound vegetation and state of erosion. For vegetation, mounds were ranked on a scale of 1-4 based on percent cover and vegetation type. Ranking criteria are displayed in table 3.1, and examples of mounds in each rank are shown in figure 3.2. Vegetation ranks were grouped this way to investigate the influence of different types and sizes of vegetation on soil chemical properties.

Table 3.1 Description of Mound Vegetation Ranking System.

Vegetation Rank	Description				
1	Bare mounds with no vegetative cover present on the mound. Some grasses may be present along the lower skirt of the mound, but the majority is completely bare.				
2	Short grasses and shrubs dominate the vegetation present, examples include <i>Solanum dulcamara</i> (evening nightshade), lantana, <i>Euphorbia</i> (spurge), <i>Hyparrhenia</i> (thatching grass), and <i>Andropogon</i> (bluestem).				
3	At least 1 tree present which is growing from within the mound. Remaining vegetation is composed of grasses or shrubs with less than 100 % mound surface cover.				
4	Mounds encompassed by a thicket of tall vegetation covering the entire mound (100 %) as well as at least 1 tree.				

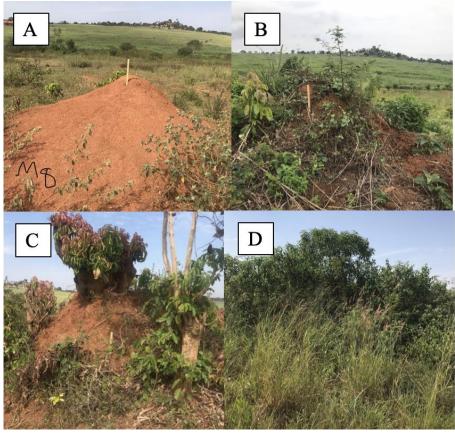


Figure 3.2 Examples of a *Macrotermes* mound from each vegetation rank, photographed at the study site by Kimberlyn M. Pace with a 30 cm ruler for scale. (A) rank 1, (B) rank 2, (C) rank 3, and (D) rank 4.

To categorize state of erosion, mounds were also ranked on a scale of 1-4. Examples of mounds in each rank are shown in figure 3.3, and description criteria are described in table 3.2. If secondary termites (physiologically distinct from *Macrotermes* termites) were observed to be colonizing abandoned mounds, then it was recorded as a separate observation outside of the ranking. Living mounds were always given a rank of 1 because every one of them showed signs of active growth, and ranks 2-4 were given to abandoned mounds in their various stages of erosion.

Table 3.2 Description of Mound Erosion Ranking System.

Erosion Rank	Description
1	Mounds which are actively being built or exhibiting net growth.
2	Mounds experiencing net erosion but are still intact structurally. There is no observable growth.
3	Mounds which are substantially eroded and/or falling apart. Extreme loss of structural integrity is observable.
4	Mounds which are highly eroded and are almost completely leveled, but underground structures (galleries, tunnels) are still present.

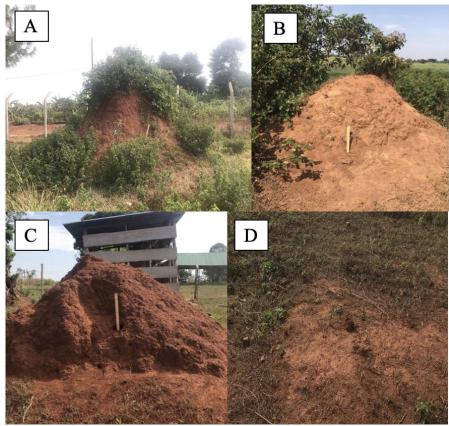


Figure 3.3 Examples of a *Macrotermes* mound from each erosion rank, photographed at the study site by Kimberlyn M. Pace, with a 30 cm ruler for scale. (A) rank 1, (B) rank 2, (C) rank 3, and (D) rank 4.

Landscape position was divided into three sections: upslope, downslope, and valley. This division was chosen based on percent slope as well as proximity to a small valley slightly past the boundary of the farm. "Upslope" refers to mounds farthest away from the valley where the slope is between 0-1 %, "downslope" refers to mounds closer to the valley bottom on a slightly steeper incline of 1-3 %, and "valley" mounds are located in the bottom of the landscape near an ephemeral stream.

Soil texture was determined to be a sandy loam using hand texturing methods.

The soil was air-dried, sifted to 2 mm, and classified according to the USDA Soil

Texture Triangle.

## **3.2.3.** Chemical Survey

A chemical analysis of 34 mounds was conducted by taking soil samples from a composite of the outer wall of each mound, as well as a more detailed spatial survey of one dead mound and one living mound. Samples taken from highly eroded mounds were taken using a composite of 4 soil cores, each of which hit at least one tunnel or gallery. The spatial survey includes samples taken from the upper and middle outer wall (0-15 cm depth), and base outer wall (0-30 cm depth), as well as samples 30 cm, 45 cm, and 60 cm deep into the center of the mound. The basis for this being that spatial variability of nutrients, pH, and microbial activity has been observed in termite mounds (Erens, 2015).

For each plot, pH and TDS values were measured using portable field meters (OAKTON pHTester5; OAKTON TDSTestr11, Vernon Hills, IL). From there, electrolytic conductivity (EC) was calculated using the following equation to estimate total dissolved charge using the TDS data:

$$EC (ds/m) = \frac{TDS (mg l-1)}{640}$$
 (Eq. 1)

Samples were tested for N, P, and K using the Palintest Soil Testing Kit (SKW 500 Complete Soil Kit, Gateshead, United Kingdom) at Jabba Engineering LTD in K'la, Uganda. To prepare the samples for analysis, all soil was air dried for 48 hours, ground, and sifted to 2 mm.

Nitrate (NO<sub>3</sub>-N) was extracted from the soil using 1M Ammonium Chloride (Extract-N) and then reduced to nitrite using Nitratest<sup>tm</sup> powder from the PalinTest soil kit. The resulting extract was then reacted with Nitricol reagent and analyzed using a photometer provided by the PalinTest Field kit. Sample containers were filled with 50 ml DI water, a scoop of extract N powder was added to each sample container using the PT 311 Long-Handled Extract-N scoop. Samples were then shaken until the powder was fully dissolved. The soil sample was added to each respective sample container using a 2 ml scoop, and then shaken for 1 minute. The sample was then filtered through Whatman Grade 40 filter paper and into a clean sample container. Ten ml of this filtered extract was transferred to a clean cuvette, and 1 Nitricol tablet was added to the filtered extract. The cuvette was then allowed to sit for 10 minutes to allow for color development. Phot 007 was selected on the SoilTest 10 photometer and the cuvette was inserted.

Extractable P was extracted using 0.5M Sodium Bicarbonate (Extract P), which is also known as Olsen extract, and then reacted with ammonium molybdate in acidic conditions. The sample containers were filled with 50 ml DI water. 5 extract P tablets were added to each sample container and shaken until dissolved. A 2 ml scoop of soil was added to the solution and shaken for 1 minute. A filter paper was placed above a clean sample container and the sample was poured through it until 2 ml was collected

and placed into a clean photometer cuvette. DI water was added to the sample until it reached 10 ml total volume. One acidifying S tablet was dissolved into the cuvette, and one phosphate P tablet was added and dissolved after that. Cuvettes were left alone for 10 minutes to allow for color development. Phot 009 Phosphate-P was selected on the photometer and the cuvette was inserted.

Extractable K was extracted using 0.1M Magnesium Acetate (Extract K), and then reacted with sodium tetraphenylboron (Potassium-K tablet) which will precipitate a white complex that is measured by photometry. First, sample containers were filled with 50 ml DI water, and then 1 level extract scoop (2.5 ml) of Extract K was added and shaken to dissolve. One 2 ml scoop of soil was added to the solution and shaken for 1 minute. A filter paper was folded and placed above a clean sample container and the solution was poured over it until 10 ml was able to be collected. The 10 ml subsample was placed in a clean cuvette and 1 Potassium-K tablet was added to the cuvette and allowed to dissolve. The cuvette was allowed to sit for 2 minutes to allow for a white complex to precipitate out of solution. Phot 009 K was selected on the SoilTest 10 photometer and the cuvette was then inserted.

#### 3.2.4. Statistical Analysis

To test for significance between different indicators and for differences in fertility, analysis of variance (ANOVA) tests were ran using proc glm, as well as Fishers Least Significant differences (LSD). Statistical analysis was performed using SAS 9.4 (Cary, NY).

#### 3.3. Results and Discussion

The objective of this section is to determine a simple visual indicator of fertility which could then be used to make recommendations to farmers about which mounds to save and apply to their field, and which to discard as usual. Can predictions be made about the probability of selecting a fertile mound based on vegetation, state of erosion, or if living termites are still present? Can high N and P be matched with position on a slope or type of vegetative cover? In this case, because application of organic amendments is often limited by labor costs, would it be worth it to walk an extra 50 meters to a mound which has a higher probability of being a greater source of fertility?

## **3.3.1. Physical Survey**

Distribution of mounds based on location, mound status, erosion rank, and vegetation rank is displayed in table 3.3. This includes both number of mounds which fall into each category as well as the percent of the total within each category.

**Table 3.3 Physical Survey Results.** 

	Number of Mounds	Percent of Total (%)
Location		
Upslope	14	41
Downslope	9	26
Valley	11	32
Mound Status		
Living	11	32
Dead	19	56
Secondary	4	12
Erosion Rank		
1	10	29
2	7	21
3	11	32
4	6	18
Vegetation Rank		
1	9	26
2	13	38
3	7	21
4	5	15

41~% of the mounds recorded were located on the level upslope part of the site landscape, where slope ranged from 0-1 %. 32 % were located in the valley, and 26 % were located in the downslope section of the terrain, on a 1-3 % slope.

Combining the dead and secondary mound categories, a majority (68 %) of the mounds on the property had been abandoned or otherwise killed and occasionally re-

occupied, compared to only 32 % of the mounds being currently occupied by the termites that constructed them.

29 % of the mounds fell into erosion rank 1, 21 % into rank 2, 32 % into rank 3, and 17 % into rank 4. Ranks 1-3 included mounds which were still visibly in-tact to some degree, whereas rank 4 was reserved for mounds which had completely eroded but evidence still existed to indicate their presence. Colonies may vacate for a multitude of reasons, however the number of years a mound has been vacant could potentially be estimated in the field by looking at the slope of the mound wall relative to the ground. As mounds grow, soil is deposited at the apex, creating very steep walls. As mounds erode, often the first part of the structure to be lost is the less solid, newer growth on top. It can be inferred that mounds with a smaller slope have been eroded for longer periods of time than mounds with steeper sloped outer walls.

A majority (65 %) of mounds were bare to partially covered with small shrubs and grasses (rank 1-2) whereas only 35 % of mounds contained trees (rank 3-4). Thicketed mounds (rank 4) were generally located downslope or in the valley, but that is most likely a result of land use. The upslope section of the study site has almost been entirely converted into farmland, with the downslope and valley section left as bush for grazing. Vegetation can be used as an indicator to estimate how long a mound has been abandoned. Depth of root penetration within the mound outer wall can be measured within the field, which serves as a qualitative estimation tool. Roots growing into the mound are a prime food source and will not be allowed to grow very deeply, and instead

be used as food for the colony. After the mound is abandoned, roots can grow as they please into the mound to greater depths.

Erosion rank differed significantly by mound type with  $\alpha$ =0.1 (P=0.0578). Vegetation rank differed significantly by mound type at  $\alpha$ =0.1, with P<0.0001. Significant differences were not observed between mound type and landscape position, type and erosion rank, or type and vegetation rank.

Table 3.4 Volume (m<sup>3</sup>) of Living and Dead Mounds by Landscape Position

	Living Mound	Dead Mound	<b>Total Position</b>		
	Volume	Volume	Volume		
_	$(m^3)$	$(m^3)$	$(m^3)$		
Upslope	6.73	8.02	14.75		
Downslope	3.70	6.28	9.98		
Valley	0.02	12.60	12.62		
Total Volume	10.45	26.90	37.35		
Average Volume	1.31	1.22			

Mound above ground volume ranged from 0-5.13 m<sup>3</sup> of accessible amendment. Mounds which had a volume of 0 m<sup>3</sup> were those which fall into erosion rank 4, where the mound has been almost entirely eroded but evidence of it still exists both above and below ground. In table 3.4, total above-ground mound volume at the study site is broken up into the three landscape positions as well as the status of the mound within each

landscape position. This is important because opportunity cost is crucial when developing recommendations for farmers.

The landscape position with the greatest volume of accessible amendment is upslope, with 14.75 m³ amendment, followed by the valley position with 12.62 m³, and lastly the downslope position with 9.98 m³ total estimated volume. A majority of the mounds were located in the upslope section, but mounds located in the valley tended to be larger.

Dead mounds contained a greater volume of available soil for use at the study site: 26.90 m³ total compared to 10.45 m³ total amendment available from living mounds. This is due to the lower density of occupied mounds on the property, and the fact that dead mounds tended to be older whereas living mounds tended to be more newly constructed and therefore smaller overall. On average, dead mounds were 0.09 m³ smaller in volume than living mounds, which is because there are no termites present inside of them to maintain structural integrity, increasing erosion. The mounds present at this study site were several cubic meters smaller than *Macrotermes* mounds present in other parts of SSA, and even in other parts of Uganda. Many of the mounds appeared to be relatively young, and many had been partially knocked down by farmers, which would influence their small size. This area is very heavily cultivated, and it has a larger percentage of sand within the soil profile due to proximity to Lake Victoria, decreasing the likelihood of large mounds in excess of 8 m³ forming.

Estimated volume of the mound can serve as an indicator for where the clay particles used to construct it were retrieved from. Younger, smaller mounds are built

using primarily surface horizon particles, from the top meter of soil. In this area, clay is most likely depleted from these layers relatively quickly as there is not much to begin with. The texture of the field site is that of a sandy loam, which by definition has less than 20 % clay. Older mounds tend to be larger because of accumulation of clay over time as the colony grows and adds more workers, with the termites burrowing deeper into the soil profile to retrieve clay particles from several meters deep, sometimes even tens of meters. The fertility associated with the different locations of clay particles could be estimated based on volume of the mound. It can be inferred then, that smaller mounds are constructed primarily of surface clay particles and larger mounds have had deeper particles incorporated into their structures.

Small mounds are sequestering soil fertility from surrounding topsoil, which is most likely to contain plant nutrients from cultivation practices and decomposing soil organisms and vegetation. Large mounds, simply by sheer size, have the potential to concentrate nutrients over time as they accumulate large quantities of plant material, dead termite skeletons, and potential fertility attached to soil particles. Living mounds which are in this mid-range are probably investing a majority of the sequestered fertility to grow their colony and establish successful fungus gardens, and dead mounds in this range were most likely abandoned or otherwise killed before they could accumulate an excess of fertility to store in the mound materials. Smaller mounds were overwhelmingly dead mounds, with twice as many dead in the 1-1 m<sup>3</sup> range and three times as many in the 0-1 m range. It is unknown what caused so many mounds to be abandoned so early,

potential causes could be pressure from farmers prior to the establishment of the research farm as well as activity from the farm itself.

# **3.3.2.** Chemical Survey

Physical indicators were used to provide insight into the chemistry of the mounds, in order to simplify management recommendations to local farmers. Because soil testing equipment is not accessible to these farmers, it is necessary to structure the chemical data based on simple, qualitative categories which farmers can easily understand. Therefore, table 3.5 displays chemistry of the outer wall of the mound based on location, status, erosion rank, and vegetation rank. This includes tested variables such as N, P, K, pH, and EC.

**Table 3.5 Chemical Survey Results.** 

	Average	Fertility in M Wall	ound Outer		Average EC	
		(mg kg <sup>-1</sup> )		Average pH	(ds m <sup>-1</sup> )	
	N	P	K			
Location						
Upslope	24.0	11.8	439.6	6.61	1.10	
Downslope	33.4	13.8	452.2	6.80	1.24	
Valley	17.2	9.6	430.9	6.75	1.16	
Mound Status						
Living	21.5	12.3	445.5	6.72	0.78	
Dead	22.4	10.2	436.0	6.71	1.24	
Secondary	40.0	16.8	445.0	6.63	1.79	
Erosion Rank						
1	22.8	10.9	450.0	6.80	0.78	
2	26.3	10.3	421.9	6.99	1.52	
3	31.0	17.4	450.7	6.43	1.14	
4	14.3	8.4	455.0	6.73	1.36	
Vegetation Rank						
1	22.0	13.4	445.0	6.51	1.60	
2	31.6	12.9	446.4	6.79	0.85	
3	29.3	10.9	427.0	6.85	1.03	
4	16.8	8.8	444.2	6.65	1.30	

At  $\alpha$ =0.05, pH did not significantly differ between mounds in regard to mound type (living, dead, secondary), landscape position or when considering interactions between landscape position and type. The pH of these mounds was around 0.8 units less

than previously recorded for *Macrotermes* mounds in Uganda, where mounds were found to be around pH 7.4 (Watson, 1977).

At  $\alpha$ =0.05, statistical differences were observed between living and secondary mounds in regards to Electrolytic Conductivity (EC) (P=0.0083), where living mounds had significantly lower EC values than mounds which had been previously abandoned and subsequently inhabited by secondary termites. There were not statistical differences observed between living and dead mounds. On average, living mounds had a EC value of 0.78 ds m<sup>-1</sup>, dead mounds of 1.24 ds m<sup>-1</sup>, and secondarily inhabited mounds of 1.79 ds m<sup>-1</sup>. As mounds age, average EC increased. This could be the result of termites no longer depleting any dissolved particles with day to day processes, or it could be due to loss of the structural integrity of the mound. Mounds are maintained to control evaporative processes and retain moisture during dry seasons. As mounds are no longer maintained, these complex systems will begin to deteriorate, allowing for increased evaporation from mound structures, leaving behind any particles which were dissolved. When secondary termites inhabit the mound once again, they are bringing substances into the mound without maintaining its structural integrity, which might explain why secondarily inhabited mounds had the highest average EC value.

At  $\alpha$ =0.05, EC did not differ between landscape position, indicating no effect of runoff or groundwater movement downslope on the accumulation of dissolved solids within the outer wall of the mound (P=0.6586). Significant interactions were observed when mound type was analyzed as a function of landscape. Pomeroy (1976a) observed similar values in Uganda, where those mounds which were not located in valley bottoms

were slightly enriched in N, P, and K. The soil P concentrations observed in this study differed from that analysis, as mounds located in the valley bottom had triple the concentration of extractable P compared to mounds located upslope.

At  $\alpha$ =0.1, N did not differ significantly by living vs. dead status (P=0.1930), landscape position (P=0.1796), erosion rank (P=0.3655), vegetation rank (P=0.7380), or when looking at interactions between erosion rank and vegetation rank (P=0.3917). P did not differ significantly living vs. dead status (P=0.4633), landscape position (P=0.7465), erosion rank (P=0.8829), or vegetation rank (P=0.7919), but did differ when looking at interactions between erosion rank and vegetation rank (P=0.0337). In this case, selecting mounds which are erosion rank 3 and vegetation rank 1 had the greatest concentration of extractable P. K did differ significantly between mounds of different status (P=0.0388), with living and secondarily inhabited mounds having greater concentrations of extractable K than dead mounds which were not secondarily inhabited. Additionally, significant differences were observed between mounds of different erosion ranks (P=0.0549), and when looking at interactions between erosion and vegetation ranks (P=0.0358). Mounds which were erosion rank 4 had the greatest concentration of extractable K, and mounds which were combined erosion rank 4 and vegetation rank 1 had the greatest average extractable K.

Mounds assigned erosion rank 4 were no longer structurally intact or even visible as a mound structure, sometimes only indicators of their historic presence indicated that there was a mound there. Meyer (1960) observed that mounds in the DRC tended to create sterile patches where vegetation did not grow. Referring to table 3.5, these

mounds also had lower N & P than surrounding mounds. Perhaps this is a contributing factor to the overall lack of vegetation in these mounds, assuming other factors also come into play which could not be tested for in this study.

N content was highest in mounds which had been secondarily inhabited by inquilines of unknown species. This contradicts a prior study of inquiline inhabited *Macrotermes* mounds which had been inhabited by the inquiline *Cornitermes Silvestri* resulted in higher losses of nitrate (NO<sub>3</sub>-N) from the mound, leading to lower overall N concentrations (Holt, 2000).

The field from which these samples were taken was left fallow for 5 years prior to the study. In the past, fertility replenishment was accomplished throughout SSA through shifting cultivation, where fields were left fallow once depleted and allowed to naturally replenish before they were cultivated again. These values recorded in the mounds are comparable to a field which had been left fallow for 5 years, indicating that while they do not compare to the fertility that the manure is adding, they can still replenish fertility lost at a comparable rate to shifting cultivation. Subsistence farmers cannot afford to leave their fields fallow for 5 years, especially when plot sizes are 0.1 ha or less. Using mound soil in this area may be a suitable replacement for this traditional practice now that population pressures have decreased the practice of shifting cultivation.

Uganda receives a significant portion (22 %) of its N fertility replenishment through atmospheric deposition, a number which is comparable to that supplied by N fixation (also 22 %) (Nkonya, 2005), which is a possible explanation for why the values

of N are greater for the 0-15 cm depth versus the 15-30 cm depth. In fact, atmospheric N deposition can account for up to 16.13 kg ha<sup>-1</sup> N annually as well as 0.80 kg ha<sup>-1</sup> P (13 %) and 3.15 kg ha<sup>-1</sup> K (20 %) (Nkonya, 2005).

The skeleton of these termites contains chitin (Redford, 1984), which is high in N. Higher termites (as well as ants and other invertebrates) sometimes pile their dead in specific locations of the mound, which could impact N distribution depending on where the soil sample was taken within the mound. N contents were highest in the bottom outer wall of the living mound, and between 15-30 cm deep halfway up the dead mound.

In regard to extractable K recorded in termite mounds, this is highly dependent on the naturally occurring subsoil clay minerals which termites are sequestering to build their mounds. It is possible that K-rich volcanic parent materials were historically deposited in this area from Mt. Elgon, located 144 km north east of the study site.

Mound building termites have been observed to transform, or biogenically weather clay minerals which they are bringing up from the subsoil. Because these particles can be sourced from up to 30 meters below the soil surface, it can be assumed that a portion of them have not been weathered as thoroughly as surface particles. In the case of Eastern Uganda, where the soil geology is heavily influenced by nearby Mount Elgon, a source of volcanic, K-rich primary silicates, which were most likely deposited in the past. This leads them to be situated at a lower depth in the soil profile, within the foraging range of the termites. Proposed by (Jouquet, 2011) using a laboratory simulation, it was shown that as termites bring these clay minerals to the surface, their saliva potentially acts as a weathering agent with the ability to increase the expandable layers of phyllosilicates.

This could also be due to the exposure of the clays to the climatic weathering processes located at the surface. The explanation they provide in their analysis proposes that as termites are processing, or grinding, these soil particles to prepare them as building materials, this mechanical process will increase the weatherable surface area of the clay particles, thereby releasing interlayer K<sup>+</sup> ions of these un-weathered, 2:1 clays into the surrounding soil solution surrounding and within mound structures. Elevated levels of K within *Macrotermes* mounds compared to surrounding topsoil have been commonly observed throughout East Africa (Mahaney, 1999), a result which is fairly consistent throughout the literature. While other mechanisms are likely also responsible for the increased K found in *Macrotermes* mounds, this could be a potential source for the mounds in this study due to the underlying mineralogy of the study area. The reasoning behind this is that the soil type is actually that of a Nitisol (Bakamanume, 2010), instead of the highly weathered oxisols common throughout the region. Nitisols tend to be less weathered and contain considerable amounts of phyllosilicate clays capable of releasing K<sup>+</sup> ions into solution as they are acted upon by termites and other weathering processes.

Additionally, newer termite mounds are constructed primarily of shallower surface particles. Once surface clay is depleted, termites will forage deeper into the soil profile to retrieve clay particles (Jouquet, 2015) which are presumably less weathered than the surface clays. Continuing to investigate the theory above, theoretically speaking the deeper soil particles are less weathered and therefore can potentially release interlayer K<sup>+</sup> into solution as they are processed by termites. This would mean that,

excluding all other factors, younger mounds should have a smaller concentration of solution K than older mounds, which are constructed out of deeper subsoil clay particles.

The purpose of the spatial distribution survey was to determine where N, P, and K were concentrated within the mound, and if selecting samples from different parts of the mound would have a different potential for having a higher fertility content. Samples were taken from the middle part of the mound with increasing depth: 0-15 cm, 15-30 cm, 30-45 cm, and 45-60 cm. Additionally, samples were taken from the apex of the mound at 0-15 cm and 15-30 cm depths, as well as from the base of the mound at a depth of 0-30 cm.

Spatial survey results indicate a non-uniform distribution of NPK concentrations throughout both the living and dead mound, displayed in table 3.6.

**Table 3.6 Spatial Survey Results.** 

_	Li	ving Mou (mg kg <sup>-1</sup> )	nd		Dead Mound (mg kg <sup>-1</sup> )			
	N	P	K	N	P	K		
Location								
Top								
0-15 cm	26.4	6	450	13.6	15	455		
15-30 cm	10.5	4	455	45.9	13	455		
Middle								
0-15 cm	11.2	6	450	4.4	9	455		
15-30 cm	0	6	420	79.2	12	440		
30-45 cm	10.5	6	425	40.2	14	455		
45-60 cm	17.9	-	445	3.6	13	440		
Bottom								
0-30 cm	45.9	0	450	55.5	-	455		

When only considering the outer wall of the mound for harvesting amendment, N content was highest for both living and dead mounds in the lower outer wall of the mound. Extractable P was also highest in the bottom skirt of the dead mound. There was no measurable P in the bottom outer wall of the living mound, it was almost entirely stored in the deepest part of the middle of the mound. K is evenly distributed throughout the outer wall of the mound.

The deepest sample taken from the middle of the mound was taken from the wall of a large fungus chamber located in the center of the mound. At this sample location, the N content of the living mound was 17.9 mg kg<sup>-1</sup>, whereas the N content at the same place in a dead mound was only 3.6 mg kg<sup>-1</sup>, the lowest value recorded in the dead

mound. Perhaps this has something to do with the presence of fungus combs, which are covered with N-rich nodules that the termites use as a food source. Several values of extractable P had to be removed due to testing error. These are denoted as "-" within the table. If a recommendation can be made based on position in the mound to harvest amendment depending on the nutrient of interest, then mounds could remain largely intact, preserving their other benefits in the ecosystem.

Results indicate that K distribution is not affected (Table 3.6). As these mounds are constructed, soil is deposited onto the top of the mound as the mound also expands outward. As the mound grows larger, more clays are being sourced from the deeper parts of the soil profile, indicating more un-weathered, potentially K-releasing clay particles would be deposited onto the top part of the larger mounds, which then would be further transferred to the outer walls cascading town the mound as it grows over time. Or, more simplistically, the hypothesis would be that larger mounds simply have an overall greater concentration of K in their outer wall, which is what can be estimated using this data. To look at this, mound volume was grouped into 4 categories: 0-1 m<sup>3</sup>, 1-2 m<sup>3</sup>, 2-3 m<sup>3</sup>, and >3 m<sup>3</sup>. K concentration was also examined as a function of mound height. The mounds on the property ranged from 0 m to 2.26 m tall. Heights were broken up into three categories: 0-1 m, 1-2 m, and >2 m. This data can be found in table 3.7. Large mounds were equally living and dead, and mid-range mounds were more likely to be dead as well when considering volume, and roughly equal when considering height. Overall a majority of the mounds on the property were dead which influences these comparisons.

A study of *Macrotermes* mounds previously conducted in Uganda reported that fertility did not significantly differ between the inner and outer parts of the mounds sampled, as well as between old and newly constructed parts of the mound (Pomeroy, 1976a).

Table 3.7 Fertility by Overall Mound Size Regardless of Status.

	Fertili	ty Concen	tration		Standard Deviation			
	N	P	K		N	P	K	
	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )		(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	
Surface Volume (m <sup>3</sup> )								
$0-1 \text{ m}^3$	22.2	9.5	434.2		21.58	6.67	38.82	
$1-2 \text{ m}^3$	27.5	9.6	449.0		17.73	10.64	12.25	
$2-3 \text{ m}^3$	17.6	21.3	455.0		7.76	15.71	0.00	
$>3 \text{ m}^3$	58.4	17.0	445.0		27.84	10.26	10.41	
Height (m)								
0-1 m	27.8	12.0	441.7		23.25	11.59	34.07	
1-2 m	17.4	10.7	435.5		16.52	6.13	26.50	
>2 m	21.1	12.7	445.0		8.61	6.51	17.32	

Table 3.7 includes fertility data from mounds which are grouped by size but not separated out into "living" and "dead" mounds. Without searching for termites, the farmer could simply approach a mound and have a reasonable idea of the fertility by asking the questions "is it large or small?" or "is it tall or short?" as they examine potential mounds for amendment. Once the farmer has decided on the relative size of the

mound, they can then look to see if it has been abandoned or not, as this could influence the probability of NPK enrichment within different size categories. The fertility data broken up into living and dead mounds is recorded in table 3.8, and the associated standard deviations are recorded in table 3.9. Due to sample sizes, some size categories had 0-1 observations and therefore had a standard deviation of 0.

Table 3.8 Fertility Results of Mound Size by Status Analysis.

		Dead Mound				L	iving Mou	nd
		N	P	K		N	P	K
		(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )		(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )
Surface Vo (m <sup>3</sup> )	lume							
	$0-1 \text{ m}^3$	15.9	8.2	435.4		38.52	14.00	432.0
	$1-2 \text{ m}^3$	27.5	8.0	450.0		0.0	0.0	0.0
	$2-3 \text{ m}^3$	20.0	13.7	455.0		10.2	44.0	455.0
	$>3 \text{ m}^3$	30.3	13.0	450.0		58.4	17.0	445.0
Height (m)								
	0-1 m	21.0	6.46	439.3		44.9	26.4	447.0
	1-2 m	15.6	12.7	448.3		20.1	7.8	416.2
	>2 m	18.5	12.5	440.0		26.4	13.0	455.0

**Table 3.9 Standard Deviations of Mound Size by Status Data (Table 3.7).** 

		Dead Mound				L	iving Mou	nd
		N P K			N	P	K	
		(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )		(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )
Surface Vol (m <sup>3</sup> )	lume							
	$0-1 \text{ m}^3$	14.30	4.48	40.88		26.94	14.00	432.0
	$1-2 \text{ m}^3$	17.73	10.64	12.25		0.00	0.00	0.00
	$2-3 \text{ m}^3$	7.37	5.03	0.00		0.00	0.00	0.00
	$>3 \text{ m}^3$	0.00	0.00	0.00		32.03	14.14	14.14
Height (m)								
	0-1 m	13.55	5.89	40.13		36.56	13.28	10.95
	1-2 m	18.75	7.03	12.11		14.70	3.30	32.24
	>2 m	10.32	9.19	21.21		0.00	0.00	0.00

K concentration increases as volume increases for mounds with  $<3~\text{m}^3$  volume, but then slightly decreases when mounds exceed 3 m³, significant at  $\alpha$ =0.1 (Pr>f = 0.0504). K concentrations in mounds between 0-1 m³ were statistically different than the other three volume categories. K concentration is greatest in mounds between 1-2 m tall, followed by mounds greater than 2 m and least in mounds less than 1 m tall, but the vales were not significant at  $\alpha$ =0.5. It seems that when selecting for K, farmers should harvest soil from mounds based on volume, selecting those which are larger than 1 m³ for the greatest probability of applying higher levels of extractable K.

Regardless of status, N content varied significantly by height but not volume at  $\alpha$ =0.05. Mounds which are 1 m tall or less had an average N content of 22.2 mg kg<sup>-1</sup>

(table 3.7), which was less than mounds 1-2  $m^3$  and <3  $m^3$ . If farmers want to select for N fertilizer, they should select mounds which are larger than between 1-2  $m^3$  and <3  $m^3$ .

P content was greatest in smaller mounds, significant in regard to volume  $(\alpha=0.05, Pr>f=0.0338)$  and height  $(\alpha=0.1, Pr>f=0.0884)$  (table 3.7). When considering amendment taken from dead mounds only, even higher significance was observed, at P=0.0001 for height and P=0.001 for volume (table 3.8). Concentrations were lowest in mounds smaller than 2 m³. Extractable P was greatest in mounds which were 2-3 m³ in surface volume, with 21.3 mg kg<sup>-1</sup> recorded overall regardless of status for that volume category (table 3.7). Following are mounds greater than 3 m³, which had an average concentration of 12.7 mg kg<sup>-1</sup> extractable P. These values were not statistically different from each other but were statistically different than the 0-1 m³ and 1-2 m³ categories, which both had concentrations less than 10 mg kg<sup>-1</sup>.

Interactions between mound type and volume, and mound type and height were examined. Statistical significance was observed in P concentrations for both volume and height interactions at  $\alpha$ =0.1 between living and dead mounds.

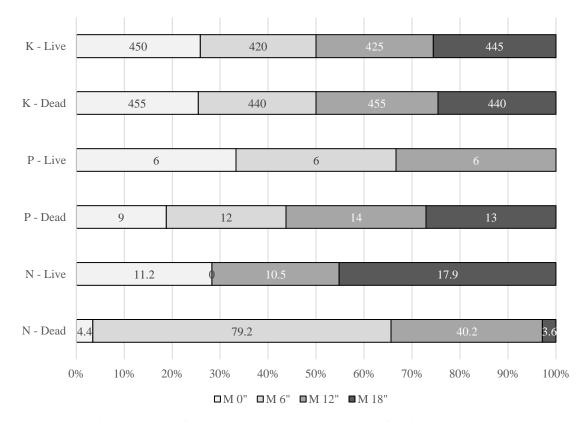


Figure 3.4 Distribution of NPK by mound depth at half-height, comparing a living *Macrotermes* mound to a dead one. Samples were taken from four depths, 0-15 cm (M 0"), 15-30 cm (M 6"), 30-45 cm (M 12"), and 45-60 cm (M 18"). Values on bars represent nutrient concentrations in each sampling depth in mg  $kg^{-1}$ .

Figure 3.4 represents K distribution within the middle of the mound sample group for the spatial survey. There were not enough samples to run statistical analysis however numeric differences will still be discussed. K distribution did not differ by depth; it was evenly distributed throughout the four sampling depths for both living and dead mounds. Extractable P recorded in the dead mound was more evenly distributed throughout all four sample locations.

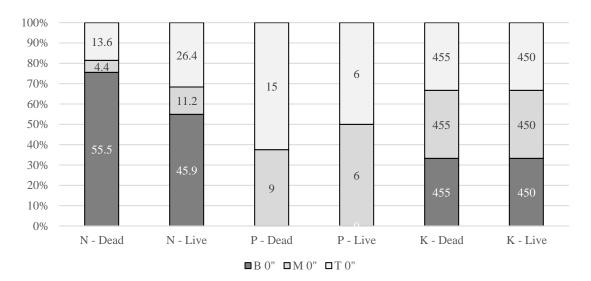


Figure 3.5 Distribution of NPK in the outer wall of a living *Macrotermes* mound compared to a dead one. Samples were taken from the bottom 0-30 cm layer (B 0"), middle 0-15 cm layer (M 0"), and top 0-15 cm layer (T 0"). Values on bars represent nutrient concentrations in each sampling depth in mg kg<sup>-1</sup>.

When considering the outer wall of the mound only, both living and dead mounds concentrated N in the bottom skirt of the mound, as shown in figure 3.5. Dead mounds concentrated P in the outer skirt, but living mounds concentrated P in the middle and top parts of the outer wall. K was evenly distributed throughout the outer wall.

When farmers are selecting a mound for amendment without destroying it, there is a greater probability of harvesting greater concentrations of soil nutrients when the amendment is taken from different locations within the mound.

#### 3.3.3. Potential as Amendment

The purpose of this survey is to evaluate the potential of termite mound soil to act as a beneficial soil amendment. The easiest management recommendation would be to harvest soil from the other wall of the mound, because it is the most accessible part. The average concentrations of NPK found in the 0-15 cm layer of the mound are displayed in table 3.10 below, compared to the top 30 cm of surrounding uncultivated topsoil.

Table 3.10 NPK Comparison of *Macrotermes* Outer Wall to Surrounding Topsoil.

Table 3:10 111 IX Con	i par ison	or mucion	cimes Outer	Wan to bu	rrounding	Topson.
	Avera	ge Concer	ntration	Standard Deviation		
		(mg kg <sup>-1</sup> )				
	N (NO <sub>3</sub> -N)	P (extract.)	K (extract.)	N	P	K
Field Soil Depth						
0-15 cm	45.5	20.6	434.6	32.35	29.25	28.08
15-30 cm	15.2	11.7	424.2	6.15	26.06	33.36
Mound Outer Wall 0-15 cm	23.9	11.7	440.1	20.53	9.67	30.39

Average concentrations of NO<sub>3</sub>-N, extractable P, and extractable K between field soil sampled from 0-15 cm, 15-30 cm and mid-height of the outer wall of 34 *Macrotermes* mounds on the farm property did differ significantly from each other at  $\alpha$ =0.05. Concentration of extractable nitrate in mound soil was statistically different

from both field depths (P <0.0001), P measured in the 0-15 cm layer of field soil was statistically different from mound soil and the 15-30 cm layer (P <0.0001), and the same was true for K (P <0.0001).

In terms of fertility, K seems to be the real benefit here. When looking at the average K of mound soil in the area regardless of life state of the mound, versus the field soil, there is a difference of 6-16 mg kg<sup>-1</sup> K for topsoil (0-15 cm depth) and subsoil (15-30 cm depth). This is likely a result of the field remaining fallow for 5 years, allowing for natural fertility replenishment. Common sources of K inflow in Uganda include composts, animal deposits from grazing, atmospheric deposition, and sedimentation (Nkonya, 2005). One tablespoon of wood ash was added at the initiation of the field study, which adds a marginal amount of soil K, however there was no interference because these cores were taken prior to the preparation of the field for planting.

The overall average N content of the termite mounds is less than the field soil at the 0-15 cm depth, but greater than the 15-30 cm depth. Soil P observed in the mounds is less than the 0-15 cm field soil depth and equal to the 15-30 cm depth. These surface soil particles have higher N contents and higher C contents than the deeper clay particles (Jouquet, 2015) most likely due to the quick turnover of plant material common throughout tropical ecosystems.

	in / histor \	A/OII	s a Fertility Amendment.			
Concentration in Outer Wall (mg kg <sup>-1</sup> )			Potential as Fertilizer			
(mg k	(g ¹)		(.	kg ha <sup>-l</sup> )		
J F	)	K	N	P	K	
.1 20			0.2		5.5	
0 10			0.0		5.5	
			0.0		5.5	
.5 0.			0.2		5.5	
.8 4.			0.3		5.5	
.8 19			0.3		5.5	
4 9.			0.1		5.5	
.2 6.			0.1		5.1	
8 10			0.0		5.5	
.7 9.			0.1		5.5	
.7 27			0.4		5.3	
.7 27			0.4		5.1	
.3 13			0.4		5.4	
.6 7.	.0	385	0.2	0.1	4.6	
.2 -	- 4	440	0.5	-	5.3	
6 6.	.0	455	0.1	0.1	5.5	
.0 6.	.0	450	0.2	0.1	5.4	
.2 44	0	455	0.1	0.5	5.5	
. 11	.0	440	-	0.1	5.3	
.5 13	3.0	455	0.3	0.2	5.5	
.6 11	.0	455	0.5	0.1	5.5	
0 8.	.0	395	0.0	0.1	4.8	
.0 24	.0	455	0.1	0.3	5.5	
.0 12	2.0	430	0.4	0.1	5.2	
9 5.	.0	300	0.1	0.1	3.6	
0.	.0	455	-	0.0	5.5	
.6 6.	.0	455	0.4	0.1	5.5	
.8 0.			0.5		5.5	
.4 13			0.3		5.5	
.3 10			0.5		5.4	
					5.4	
					5.5	
					5.3	
					5.0	
	.5 1. .0 7. .8 30	.5 1.0 4 .0 7.0 4 .8 30.0	.5 1.0 450 .0 7.0 455 .8 30.0 435	.5 1.0 450 0.2 .0 7.0 455 1.0 .8 30.0 435 1.0	.5 1.0 450 0.2 0.0 .0 7.0 455 1.0 0.1 .8 30.0 435 1.0 0.4	

Table 3.11 displays concentrations of NO<sub>3</sub>-N, extractable P, and extractable K measured in 34 Macrotermes mounds in Kyando, Uganda. These values have been used to calculate fertility added per planting hole if that mound had been used as amendment at the application rate used in this study. This table is useful when considering the suitability as amendment of the different mounds located at the study site. In general, N content ranged from 0-85.8 mg kg<sup>-1</sup>, P ranged from 0-30 mg kg<sup>-1</sup>, and K ranged from 300-455 mg kg<sup>-1</sup>. Data entries of "-" indicate outliers which were due to testing error. The mounds that were used as amendment were on the lower end of the range for N and P, and on the high end for K; Mound 7 was used as "dead termite mound soil" and mound 8 was used as "living termite mound soil". Using the application rate and planting spacing used in this study, there are 2,828 planting holes per 0.1 ha, with 28,280 planting holes per ha. Assuming that roughly 0.4 kg termite mound soil is being applied per planting hole, the kg ha<sup>-1</sup> NPK fertilizer rates were calculated. These rates ranged from 0 kg ha<sup>-1</sup> to 1.1 kg ha<sup>-1</sup> N, 0 kg ha<sup>-1</sup> to 0.4 kg ha<sup>-1</sup> P, and 4.6 kg ha<sup>-1</sup> to 5.5 kg ha<sup>-1</sup> K. The overall N balance observed in Uganda is -48.02 kg ha<sup>-1</sup>, P is -10.8 kg ha<sup>-1</sup>, and K is -51.09 kg ha<sup>-1</sup> (Nkonya, 2005).

The addition of termite mound soil as another nutrient inflow to the system could offset these negative balances to varying degrees depending on location. Because fertility is stored within the mound until it is knocked down or otherwise eroded, the addition of destroyed mounds back into the soil could provide a small inflow and speed up the nutrient cycling in the area. Local nutrient outflows for rural Uganda were calculated by Nkonya (2005). The local mean N outflow from exported crop residue

(removal of crop biomass without re-incorporation into the soil) is 1.18 kg ha<sup>-1</sup>, which is comparable to the maximum potential N added as fertilizer through the addition of termite mound soil (1.1 kg ha<sup>-1</sup>), as is exported animal products, which average at 0.74 kg ha<sup>-1</sup>. Potential additions of extractable P from termite mound soil maxed out at 0.4 kg ha<sup>-1</sup> in this study. Local P outflows which this could compensate for include exported crop residues (0.1 kg ha<sup>-1</sup>) and exported animal products (0.26 kg ha<sup>-1</sup>) (Nkonya 2005). Termite mound soil applied as fertilizer can reach a maximum of 5.5 kg ha<sup>-1</sup> K according to this study, which can compensate for several documented K outflows for the area. These include exported crop residues (0.96 kg ha<sup>-1</sup>), exported animal products (0.27 kg ha<sup>-1</sup>), leaching losses (0.91 kg ha<sup>-1</sup>), and soil erosion losses (3.18 kg ha<sup>-1</sup>) (Nkonya, 2005). If visual indicators are used to select mounds with high probability of these upper range values, these nutrient outflows may be compensated for by the addition of termite mound soil.

Recommendations should be as simple as possible for increased and sustained adoption. Therefore, only one or two visual indicators should be included in the recommendation, without any interactions. Based on this data, mound size and life state are good candidates as primary indicators of fertility. Other decent indicators include Any recommendations generated from this data set are highly site specific and would require modification before being applied elsewhere due to variability in mound chemistry, soil chemistry and mineralogy, and differing cultural practices. The conclusions drawn using the data from this study are suited for rural Ugandan farmers who live in the eastern Lake Victoria Crescent, near an abundance of abandoned

Macrotermes mounds which are moderately vegetated and are still structurally in-tact. Before recommending the practice of using Macrotermes soil as a field amendment to a population, mound soil should be evaluated for fertility, pH, and EC to determine whether the visual indicators used in this study can also accurately gauge soil fertility in the area of interest, especially if the underlying soil mineralogy and geology differs heavily from that of Eastern Uganda. Plant available fertility sequestered by these termites varies highly between sites and so further testing needs to be done before a set of universal application guidelines can be established for the use of Macrotermes mound soil as an amendment. This study poses additional questions as well as adds important data to the growing catalog of chemical and physical data available on the usefulness of Macrotermes mound soil as an agricultural input in a specific district of Eastern Uganda, where alternative solutions are necessary to address growing sustainability issues.

When selecting a mound to use as a soil amendment, there are several indicators which can be used to increase the likelihood of selecting a mound with greater concentrations of NPK. These included mound height, estimated volume, position within a landscape, status, erosion rank, and vegetation rank.

For sustainability purposes, farmers should preferentially select dead mounds to use as amendment due to the positive influence higher termites have on savanna ecosystems. If termite populations are decreased, nutrient balances and natural soil C and N cycles could be disturbed, as well as vegetation patterns which would in turn influence behavior of grazing mammals, insects, etc.

Mound size is an important indicator both in terms of probability for higher fertility. Larger mounds are likely being taken down at higher rates already by farmers due to their influence on nearby fields. Larger mounds (in terms of height and volume) should be selected to use as amendment when the choice is between a large mound and a new, small mound. Termite mound soil is also occasionally used as building material, presumably due to the high clay content of the mound (Debelo, 2015). This could be a proposed alternate use of the mound soil if proven to be infertile or detrimental to crop production which could be encouraged instead of disposal away from the field.

## 3.4. Conclusions

Termites provide many beneficial ecosystem services which are essential to both broad range ecosystem functioning as well as cultivation, as evidenced by the Zaï farming in Burkina Faso (Kaiser, 2017). There is benefit to leaving some mounds in-tact while harvesting others for amendment, and the importance of this should be communicated alongside other management recommendations. Removing and disposing of a mound, especially one which is well established, takes time and energy which could be saved and instead used in the field.

Recommendations to Ugandan subsistence farmers who wish to use this amendment include several visual indicators which may be used with moderate accuracy to predict whether a mound should be used as amendment. These indicators include mound size (height and volume), erosion rank, vegetation rank, and status. Depending on the nutrient of interest, different indicators may be used. If selecting for N or P,

farmers should first try to use amendment from mounds which are less than 1 m tall. Additional P indicators are mounds which are erosion rank 3 which are modestly eroded abandoned mounds, or vegetation rank 1, which are bare. If selecting for K, farmers should choose mounds which are either living or have been abandoned but are inhabited by secondary termites. They may also select mounds which are both heavily eroded (rank 4) and bare (rank 1). They may also select mounds less than 3 m<sup>3</sup> in volume or greater than 2 m tall.

Using termite mound soil as amendment should be considered alongside other sustainable farming techniques when management strategies are being developed for different communities. Termite mound soil is a beneficial amendment and should be evaluated and recommended alongside techniques such as crop rotation, reducing tillage, using mulches, and applying manures. This amendment is a suitable first step for subsistence farmers to begin restoring their soil with no financial investment. The addition of soil harvested from a dead termite mound replaces some fertility lost to cultivation while allowing farmers to continue farming without sacrificing any growing seasons to leave their fields fallow, as they invest in other potential sources of income and eventually gain access to other agricultural inputs such as animal manures and inorganic fertilizers.

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# 4. APPLICATION OF MACROTERMES MOUND SOIL AS A SOIL AMENDMENT FOR MAIZE IN EASTERN UGANDA

#### 4.1. Introduction

There are an estimated 254 million food insecure people in Sub-Saharan Africa (SSA), and this number is expected to rise in the coming decade due to pressures from increasing populations (Ittersum, 2016; Rosen, 2015; Tittonell, 2013) and urbanization without agricultural growth (Djurfeldt, 2015). Roughly 33 % of people in SSA are classified as undernourished, with greater than 60 % of these people living in East Africa (Khan, 2014). Long term food security cannot be achieved without first supporting and empowering smallholder farmers to be more self-sufficient by increasing access to resources that are available to them (WDR, 2008). Because of the high financial cost and lack of availability in rural communities, improved cultivars and inorganic fertilizers are not a suitable solution for many farmers. Alternative solutions need to be investigated to address the decline in food production per capita that farmers in many parts of Africa are experiencing.

This decline in food production capacity is primarily the result of a general decline in soil fertility. Soils are becoming more depleted every year as farmers are unable to replace the nutrients lost to cultivation during the growing season (Bedada, 2014). Maize, a staple grain crop grown throughout much of SSA, has a high nutrient demand and will thoroughly strip the soil of accessible fertility. Historically, farmers throughout SSA practiced slash and burn agriculture, moving on to uncultivated fields as

land was depleted of nutrients (Bedada, 2014; Henao, 2006). By allowing land to remain fallow following depletion, natural nutrient cycling was able to replenish soil fertility over time. Population pressures increased substantially, necessitating the need to transition to permanent cultivation. Because farm sizes are often less than 0.1ha, many farmers do not have the ability to leave sections of their fields fallow for even 1 growing season. The average annual depletion rate for 37 countries in SSA is around 22 kg N, 2.5 kg P, and 15 kg P ha<sup>-1</sup> (Smaling, 1997). In Uganda, N balances are -48.02 kg ha<sup>-1</sup>, P as -10.8 kg ha<sup>-1</sup>, and K as -51.09 kg ha<sup>-1</sup> (Nkonya, 2005). Compared to the overall smallholder farm application rate of N fertilizer averaged over all of SSA, which is 13 kg ha<sup>-1</sup> (Njoroge, 2017), Ugandans are applying some of the lowest rates of inorganic fertilizer in SSA. Average maize yields are also a reflection of these values; on average, rain fed maize yields in SSA fall between 1.2-2.2 tons ha<sup>-1</sup>, 15-27 % of the total potential yield (Njoroge, 2017), whereas maize yields from East Ugandan farms surrounding the study site were as low as 350 kg ha<sup>-1</sup>, which is around a quarter of the overall average for SSA.

These issues could be addressed with moderate application of mineral fertilizers, but many countries throughout SSA cannot economically justify inorganic fertilizer manufacturing facilities and infrastructure due to low domestic demand (Quiñones, 1997; Sánchez, 2005). Transportation infrastructure, or lack thereof, is also a contributor. A majority of the subsistence farmers which are most in need of fertilizers are located far away from major city centers and are very spread out geographically with no way of transporting themselves to large markets (Bedada, 2014). As of 2007, Uganda

had a rate of 2 kg inorganic fertilizer applied per hectare (FAO, 2007), where only 10 % of smallholder farms were observed applying any at all (Pender, 2001). The NARO and FAO estimate from 1999 claim that the mineral NPK application rate in Uganda is even lower at 1 kg ha<sup>-1</sup> (NARO, FAO, 1999).

Organic amendments, such as manures and composts, are commonly recommended to farmers who cannot afford improved seed or mineral fertilizers. They are less expensive, but also less nutrient dense than inorganic fertilizers and so a much greater volume of the amendment is needed. The nutrients are also not always present in readily available forms, requiring further decomposition by soil decomposers (Okalebo, 2007). Despite this, Okalebo (2007) observed that in East Africa, maize yields on smallholder farms could be raised from below 0.5 t ha<sup>-1</sup> to 3-5 t ha<sup>-1</sup> through the use of organic inputs, generally manure and composts.

The underlying issue is that many farmers still do not have sufficient access to these organic amendments, and are often discouraged when they are recommended as alternatives. Access to manure requires an investment in livestock or other small animals such as poultry or goats. Acquisition of enough animals to produce a practical amount of manure is a luxury that many people cannot afford (Quiñones, 1997; Tittonell, 2013). It is often observed that any organic amendments such as small quantities of manures or kitchen scraps are applied to small home gardens instead of maize fields, which can sometimes be located a significant distance away. Without proper transportation tools, such as wheelbarrows or carts, transporting large quantities of manure is not a feasible task for many farmers. In addition, many animals (if present at all) are not kept in closed

pens but are instead allowed to roam. Collection of manure from free range chickens and goats is not practical and therefore not a suitable amendment when the home and family maize field is not located on the same plot of land. Lastly, any manure that the farmer manages to collect will often preferentially be used as cooking fuel in rural areas. Composts are more available than manures, but most farmers do not have access to the proper equipment required for effectively incorporating residue back into the soil when it is un-composted. The application of biomass, or mulch, in the form of cut grasses or crop residue poses a risk for attracting litter feeding termites, causing many farmers to remove all biomass and dispose of it or repurpose it off-site. Farmers also may burn all biomass as a method of removal, as observed with wheat farmers in Kenya (Okalebo, 2007). Crop residues and biomass can also be used as cooking fuel, building materials (in the case of cut grasses), or livestock fodder/feed when applicable (Mtambanengwe, 2005).

One solution to the need to improve soil fertility throughout SSA could be the use of soil harvested from termite mounds. This resource is beneficial to subsistence farmers because it is a public organic resource, requiring no financial investment.

Farmers need only live near a mound to take advantage of the amendment. Many mounds are already being taken down and disposed of away from cultivated fields (Erens, 2015), so direct application to cropped soils is a better use of this resource. Many locals consider the mound-building termites pests, and a change in cultural perspective would need to occur in order to see widespread adoption of this practice, but it could prove invaluable to farmers who have limited access to other potential amendments.

To construct these mounds, termites build extensive tunnel systems extending upnto 10-12 m deep into the soil (Wood, 1988) to select wet, clay-sized soil particles, cementing them into tunnel and chamber walls (Paul, 2015). According to previous chemical analyses of the mound soil, plant essential soil nutrients are commonly concentrated within the lower central zone of most mounds (Erens, 2015), specifically N, P, K, Calcium, Magnesium, and S (Erens, 2015; Hesse, 1955; Pomeroy, 1976a; Sarcinelli, 2009; Sileshi, 2010; Watson, 1977). Of particular interest is available P, where high concentrations have been observed within mounds compared to surrounding topsoil (Rückamp, 2009). A chemical analysis of *Macrotermes* mounds in 1977, using the anion exchange resin method, reported that the mounds contained 37 mg kg<sup>-1</sup> of available P in the mounds, compared with 10 mg kg<sup>-1</sup> in the surrounding topsoil Ah horizon and 5 mg kg<sup>-1</sup> in the Ap horizon (Watson, 1977), and a 2010 study of *Macrotermes* mounds in Kenya reported that there was 20 % more available P in mounds than in surrounding topsoil (Sileshi, 2010). These nutrients are either brought up from subsoil horizons with the clay particles used for construction, or they are released as plant material is digested within the mound.

Watson (1977), after studying *Macrotermes falciger* mounds, concluded that the mounds were a beneficial amendment to cultivated soils through incorporation of the mound soil into the topsoil of the field. He observed increased concentrations of N, P, Calcium, and Magnesium in these mounds. Similarly, he also observed increased concentrations of Calcium, Magnesium, and available N in *Macrotermes* mounds from Rhodesia when compared to surrounding topsoil. Pomeroy (1976a) recorded increased

concentrations of N, P, Calcium, and K in Ugandan *Macrotermes* mounds. The usefulness of this resource has not been thoroughly evaluated in Eastern Uganda in the current century. Therefore, a field study was conducted to investigate the effects of applying termite mound soil to a crop of maize over 1 growing season.

The first objective of this chapter is to directly study the impact of applying termite mound soil as an amendment to a field of maize located on a working conservation farm in Eastern Uganda. Secondly, if it does have a significant impact on yield when compared to non-amended soil, does the life state of the mound have a significant impact?

## 4.2. Methods

# **4.2.1. Study Site**

This study was conducted at the Vocare Ministries Ltd. teaching farm located in Kyando, Mayuge District, Uganda, located in the eastern side of the Lake Victoria Crescent, over one growing season. The site is approximately 7 km northeast from the shore of Lake Victoria at 00°25.826N, 033°26.013E. Vocare Ministries is a local non-profit which imparts business skills and sustainable farming techniques to the town of Jinja and the surrounding communities. The teaching farm was developed 5 years ago on 8.9 hectares of traditionally farmed land next to the Bishop Hannington Memorial Site. There are 34 termite mounds on the property.

The ecosystem type is grouped tree savanna, with a bi-annual rainy season, although the area receives occasional showers throughout the dry seasons due to

proximity to Lake Victoria. This indicates the potential for two successful growing seasons per year depending on rainfall intensity, March-May and September-November. On average, it rains on this site 160-170 days per year, with rainfall of moderate intensity at 1500-1750 mm annually (Bakamanume, 2010). Average temperatures do not fluctuate much and generally remain around 24°C during the dry season and 22°C during the rainy season. Elevation is approximately 3800 ft above sea level (World Bank, 2016).

Prior to this study, this field had been fallow for 5 years, and before that, traditionally cultivated. It is unknown what crops had been previously grown, the most likely use was to grow local varieties of sweet potato, cassava, coffee (*Robusta*), common beans (*Phaseolus vulgaris*), or maize. Free range grazing of cattle, goats, and chickens is also a potential prior land use, but is less likely.

The soil order where the field trial is located is likely a Nitisol (sharing qualities with USDA soil orders Alfisol and Ultisol), which are deep clays common in Mukono, Jinja, Mbale, Iganga, and Tororo districts. These are moderately weathered soils with subangular blocky or granular structure (Bakamanume, 2010), and are often fertile and suitable for crop production if properly managed.

#### 4.2.2. Field Trial

The effects of soil obtained from a live termite mound, a dead termite mound, and wet cattle manure on both maize yield and root development were examined. The termite mound soil and the cattle manure was obtained from the farm property. The living and dead termite mound soil used as amendment was composited from the middle, lower, and upper outer wall of the respective type of mound. The application rate for all amendments is 350 ml per plant, a quantitative estimate of 'a handful', which is the currently taught organic amendment application method in the area. This has also been observed as an organic amendment application method by Probert et al. (1992).

Chemical analysis of the cattle manure used as amendment at the time of planting was not able to be obtained. The manure was harvested from cattle which were grazed on the farm property as well as supplemented with *Moringa oleifera*, which is also grown on the farm property. A chemical analysis of cattle manure harvested from Zimbabwe was found to contain 1.1 % N, 0.18 % P, 0.64 % K, 0.20 % Ca, and 0.08 % Mg (Zingore, 2008), which can be used as an estimate of the chemical composition of this manure given the similar ecosystem type and grazing style. The concentrations of NPK present in the living termite mound soil and the dead termite mound soil used as amendment can be found in table 4.1.

Table 4.1 NPK Added as Amendment in the Field Trial.

	Concentration (mg kg <sup>-1</sup> soil)			A	Application Rate (kg ha <sup>-1</sup> )		
_	N	P	K	N	P	K	
Treatment							
Live Soil	11.2	6.0	425.0	0.14	0.07	5.14	
Dead Soil	4.4	9.0	455.0	0.05	0.11	5.50	
Dead Soil	4.4	9.0	455.0	0.05	0.11	5.50	

The field is drip irrigated with on-site well-water and has been fallow for 5 years; before that it was traditionally farmed by local farmers. While drip irrigation is not available to many subsistence farmers, it was used in this study to eliminate some experimental error. The layout is an RCBD with 3 blocks, and 12 plots total. The field size is 142.56 m² (0.01 ha), planting hole spacing is 60 cm, and there are 2 plants in each planting hole. Therefore, planting density is 5.6 plants per m². Three seeds were initially planted in each hole and thinned to 2 plants at the 21-day mark.

Planting holes were designed as follows. Holes were dug to 15 cm deep with a hand hoe. A teaspoon of wood ash was then applied to the hole, followed by 350 ml amendment on top of that. The hole was covered with soil to a 5 cm depth and seeds placed in a line at the top of the hole, which were then covered with soil until it was level with the surface. Planting holes were marked using a string with crimped bottle caps at each increment, which were measured using 60 cm long pre-cut measuring stick. The prepared field was then covered with cut brush for soil moisture retention, harvested from the border of the field. This application method was chosen instead of

other application methods such as surface application or strip tilling in an attempt to adhere to local sustainable farming methodologies which are already being taught by local agricultural non-profits. Using application methods which are already familiar to farmers may increase adoption rates of applying termite mound soil as an amendment.

The field trial was initiated on August 20<sup>th</sup>, 2018 and concluded on January 4<sup>th</sup>, 2019, for a total of 137 days between planting and harvest. The maize was field-dried following physiological maturity until time of harvest: roughly 6 weeks.

pH and TDS values were measured using portable field meters (OAKTON pHTester5; OAKTON TDSTestr11, Vernon Hills, IL). Fertility tests to assess the mounds at each site were done including N, P, and K. This was carried out by the soil testing lab at Jabba Engineering Ltd., located in K'la, Uganda, for both field and termite mound soil. Fertility analysis was done by extraction using the Palintest Soil Testing Kit (SKW 500 Complete Soil Kit, Gateshead, United Kingdom).

The texture of the termite mound and field soil was determined using hand texturing methods and the USDA Soil Texture Triangle. Soil classification information was inferred using a local soil characterization map prepared by the National Agricultural Research Organization (NARO) of Uganda as well as soil classification resources written by the United States Department of Agriculture, Natural Resource Conservation Service (USDA, NRCS).

While planting the maize, evidence of a termite mound was observed below the soil surface. Sections of chambers and tunnels, pieces of decayed fungus, and the presence of secondary termites were three qualitative observations. In order to better

understand the location and zone of potential influence of this mound in the field, a soil texture survey was conducted (Figure 4.1). Samples were taken from the 0-15 cm layer as well as the 15-30 cm layer. Hand texturing revealed that the soil texture for the topsoil was a sandy loam across the whole field, but that it changed from a sandy loam to a sandy clay loam in the 15-30 cm layer when in the presence of the observed chambers and decayed fungus. Therefore, 6 cores were taken per plot by taking 3 cores in between each planting row, for a total of 60 cores total. They were then divided into 0-15 cm and 15-30 cm and hand textured to estimate the location of the mound based on this characteristic.

х	X	X	X	X	X	X	X	X
X	X	X	x	X	X	X	X	X
х	X	X	х	x	X	o	x	х
x	X	X	x	X	o	o	o	x
х	X	o	o	o	o	o	o	o
x	X	o	o	o	o	o	o	x
х	х	o	o	o	0	0	Х	o
X	o	o	o	o	o	o	o	X

Figure 4.1 This diagram represents soil core locations as well as the estimate of the location of the mound, where (o) represents a soil texture change in the 15-30 cm soil layer from sandy loam to sandy clay loam, indicative of the mound presence, and (x) represents where the soil texture represented that of the surrounding sandy loam topsoil.

Samples were tested for N, P, and K using the Palintest Soil Testing Kit (SKW 500 Complete Soil Kit) at Jabba Engineering LTD in K'la, Uganda. To prepare the samples for analysis, all soil was air dried for 48 hours, ground, and sifted to 2mm.

Nitrate (NO<sub>3</sub>-N) was extracted from the soil using 1M Ammonium Chloride (Extract-N) and then reduced to nitrite using Nitratest<sup>tm</sup> powder from the PalinTest soil kit. The resulting extract was then reacted with Nitricol reagent and analyzed using a photometer provided by the PalinTest Field kit. Sample containers were filled with 50 ml DI water, a scoop of extract N powder was added to each sample container using the PT 311 Long-Handled Extract-N scoop. Samples were then shaken until the powder was fully dissolved. The soil sample was added to each respective sample container using a 2 ml scoop, and then shaken for 1 minute. The sample was then filtered through Whatman Grade 40 filter paper and into a clean sample container. Ten ml of this filtered extract was transferred to a clean cuvette, and 1 Nitricol tablet was added to the filtered extract. The cuvette was then allowed to sit for 10 minutes to allow for color development. Phot 007 was selected on the SoilTest 10 photometer, and the cuvette was inserted.

Extractable P was extracted using 0.5M Sodium Bicarbonate (Extract P), which is also known as Olsen extract, and then reacted with ammonium molybdate in acidic conditions. The sample containers were filled with 50 ml DI water. 5 extract P tablets were added to each sample container and shaken until dissolved. A 2 ml scoop of soil was added to the solution and shaken for 1 minute. A filter paper was placed above a clean sample container and the sample was poured through it until 2 ml was collected and placed into a clean photometer cuvette. DI water was added to the sample until it reached 10 ml total volume. One acidifying S tablet was dissolved into the cuvette, and one phosphate P tablet was added and dissolved after that. Cuvettes were left alone for

10 minutes to allow for color development. Phot 009 Phosphate-P was selected on the photometer and the cuvette was inserted.

Extractable soil K was extracted using 0.1M Magnesium Acetate (Extract K), and then reacted with sodium tetraphenylboron (Potassium-K tablet) which will precipitate a white complex that is measured by photometry. First, sample containers were filled with 50 ml DI water, and then 1 level extract scoop (2.5 ml) of Extract K was added and shaken to dissolve. One 2 ml scoop of soil was added to the solution and shaken for 1 minute. A filter paper was folded and placed above a clean sample container and the solution was poured over it until 10 ml was able to be collected. The 10 ml subsample was placed in a clean cuvette and 1 Potassium-K tablet was added to the cuvette and allowed to dissolve. The cuvette was allowed to sit for 2 minutes to allow for a white complex to precipitate out of solution. Phot 009 K was selected on the SoilTest 10 photometer and the cuvette was then inserted.

## **4.2.3. Data Collection**

The data collection for this study was split up into two sections. The first is August 14-29, 2018, and the second is December 31, 2018-January 23, 2019. In August, the field trial was initiated and initial soil samples of both the field and the termite mounds were collected and stored. Samples from a survey of mounds on the 8.9 ha property were collected along with the spatial survey. In January, the plots were harvested and any remaining data was collected. This includes total dry biomass and grain mass per plot,

root washing and imaging, soil sampling, and AMF staining and imaging. Soil samples for the termite mounds and field were submitted to the soil testing lab in Kampala.

Grain was harvested by removing dried cobs from the middle row of each plot separately and then harvesting the rest of the plot. Cobs were husked prior to removal from the stalk to make biomass collection easier. Cobs were dried separately in the sun for three days, ginned, and then weighed on a food scale in grams (Taylor Digital 11 lb Glass Kitchen Scale, model 3807BK21, Oak Brook, IL), accurate to 2 decimal points. These readings were then converted to kilograms for analysis. Total plot yields were ultimately used for analysis instead of only middle row yields because of lack of edge effects due to the chosen amendment application method. Cob length was recorded in centimeters and averaged for 10 cobs per plot, and total number of cobs per plot were recorded to calculate average grain mass per cob. Whole stalks (with husks attached) were collected and weighed on a hanging kilogram scale, accurate to 2 decimal points, and then that value was added to the weight of the empty cobs post ginning to determine total biomass per plot. Four maize plants were randomly selected per plot for root architecture comparison & fine root subsampling. Root systems were excavated by digging in a 20 cm radius around the plant and then soaking in well water for 24 hours. Soaked roots were washed with tap water to remove soil and rock particles, photographed, and then fine roots were subsampled for AMF analysis.

# 4.2.4. Arbuscular Mycorrhizal Fungi (AMF)

Fine roots from four maize root systems per plot were subsampled and stored in a solution of 80 % ethanol and 20 % isopropyl alcohol for 24 hours prior to AMF analysis.

For clearing, roots were washed with DI battery water to remove any soil particles, then boiled on a propane stove in a 10 % Potassium Hydroxide (KOH) solution (10:90 KOH:DI Water) for 13 minutes, allowed to cool to room temperature. The roots were then stained using a 5:95 solution of black ink (Pelikan blue, Herlitz PBS AG Company, Germany) and distilled white vinegar by heating to 95°C for 3 minutes and then rinsing in DI water. The stained roots were then boiled in DI water with several drops of vinegar for 20 minutes, cooled, and observed under 10x magnification using an Amscope microscope (Biology Science Metal Glass Student Microscope, SKU M158C, Amscope, California). Images were taken using an iPhone 7 camera and analyzed for AMF infection on the same device. 35 total 0.5 cm root segments were photographed per plot, for a total of 140 segments per treatment.

To determine AMF infection density at maturity, total number of vesicles, arbuscules, and density of hyphae per unit root area, a process based on the method described by Suharno et al. (2017) was used, where the percent root infection from fungal structures will be evaluated using ratings ranging from 0-4 based on density of infection and types of fungal structures present, descriptions outlined in table 4.2.

Table 4.2 Ranking System Used to Describe AMF Colonization of Maize Roots.

AMF Rank	Description
0	No mycelium/hyphae, vesicles, or arbuscules present in the root segment.
1	Trace to 20 % mycelium/hyphae present.
2	20-60 % mycelium/hyphae.
3	>60 % hyphae present and <10 % vesicles and/or arbuscules present.
4	>40 % vesicles/arbuscules and mycelium.

Percentages expressed in this rating system are a reflection of the area root segment containing said fungal structures. Total number of vesicles and arbuscules per root segment were also recorded. Percent of hyphae and fungal structures were estimated by dividing root segment image into 10 sections and recording the number of sections containing AMF.

From there, equation 2 was used to summarize the rating data collected to compare treatments based on overall AMF infection.

% root infection = 
$$\frac{Sum \ of \ Ratings}{Maximum \ Possible \ Sum \ of \ Ratings} x \ 100\%$$
 (Eq. 2)

The AMF data collected is used as a gauge to make assumptions about the robustness of the microbial community living in each treatment area, as well as determine the effects of applying termite mound soil on AMF colonization of maize roots.

# 4.2.5. Statistical Analysis

To test for significance between treatments in terms of grain yield, biomass, and AMF percent between the four treatments, ANOVA tests were ran using proc glm, as well as Fishers LSD. Statistical analysis was performed using SAS 9.4 (Cary, NY).

## 4.3. Results and Discussion

# **4.3.1. Field Chemistry**

Soil fertility was assessed in the 0-15 cm layer and the 15-30 cm layer, with values displayed in table 4.3. NPK concentrations tended to be greater in the 0-15 cm layer when compared to the 15-30 cm layer, with the exception of a couple plots.

Table 4.3 NPK Values Recorded per Plot.

-	0-15 cm g kg <sup>-1</sup> )	K	-	15-30 cm g kg <sup>-1</sup> )	
N	P	K	N	Ъ	
			11	P	K
52	30	455	12	0	345
25	8	455	11	5	420
81	0	455	12	0	375
9	210	435	6	94	435
119	55	380	16	3	455
14	7	455	16	3	435
58	93	455	27	3	435
23	6	455	13	7	415
32	0	440	24	6	440
32	21	440	23	9	455
29	0	395	12	4	455
73	6	395	12	6	425
	25 81 9 119	25 8 81 0 9 210 119 55 14 7 58 93 23 6 32 0 32 21 29 0	25 8 455 81 0 455 9 210 435 119 55 380 14 7 455 58 93 455 23 6 455 32 0 440 32 21 440 29 0 395	25       8       455       11         81       0       455       12         9       210       435       6         119       55       380       16         14       7       455       16         58       93       455       27         23       6       455       13         32       0       440       24         32       21       440       23         29       0       395       12	25     8     455     11     5       81     0     455     12     0       9     210     435     6     94       119     55     380     16     3       14     7     455     16     3       58     93     455     27     3       23     6     455     13     7       32     0     440     24     6       32     21     440     23     9       29     0     395     12     4

Lack of growing grain for 5 years previous to this study might have something to do with those levels of N and P compared to the national average, but it is expected that a field which hadn't been fallow would have lower levels of both N and P and would most likely show a different yield result in this practice.

Soil N values were higher overall in the 0-15 cm layer than the 15-30 cm layer, which could be due to a variety of reasons. Seeing as the field was covered in shallow rooting grasses prior to this study, deposition from root exudates and biomass provided a sufficient source of organic carbon to support the microbiome of the surface, paired with sufficient soil aeration and moisture due to being left unplowed with soil cover for 5

years. This microenvironment might have contained N fixing free living microorganisms or symbiotic N fixing organisms depending on plant species present (this data was not collected). Additionally, decomposing microorganisms are present in higher numbers in the upper soil layers and favorable conditions could stimulate their activity, resulting in an increase of N mineralized in this upper soil layer. Also present are increased populations of soil organisms such as termites, other insects, and earthworms. There was significant compaction present in the 15-30 cm layer from prior land use, where soil was tilled to 30-45 cm every growing season. Compaction decreases the available pore space in the soil, thereby limiting the air and water available for microorganisms to decompose organic material. Less nutrients will be mineralized in these conditions, which is observed in the data.

Table 4.4 pH and EC Values Recorded per Plot.

		Treatment	pН	EC (ds m <sup>-1</sup> )
Plot ID	_			· · · · · · · · · · · · · · · · · · ·
	1	Control	6.5	0.36
	2	Live Termite Soil	6.4	0.29
	3	Dead Termite Soil	6	0.37
	4	Manure	3.5	2.07
	5	Manure	6.4	0.2
	6	Dead Termite Soil	6.4	0.22
	7	Control	6.2	0.32
	8	Live Termite Soil	6.6	0.23
	9	Manure	6.8	0.19
	10	Live Termite Soil	4.6	1.48
	11	Dead Termite Soil	6.8	0.22
	12	Control	3.4	2.14

There was a strong correlation between EC and pH, shown in table 4.4. The average pH of the field topsoil is pH 5.8, which is slightly acidic. Plots 4, 10, and 12 were especially acidic, between pH 3.4-4.6. This could be due to prior land use, but the exact cause is unknown. It is interesting to note that when looking at the map of the remnant termite mound in the field, these plots occur on the boundary of the mound. These low pH values correlate with high EC values observed in the same plots, with values ranging from 1.48-2.14 ds m<sup>-1</sup>, significantly higher than the average field EC which was 0.67 ds m<sup>-1</sup>. EC of the field soil falls within the normal range for typical nonsaline soils (>0-2 ds m<sup>-1</sup>) for 10 plots, with 2 plots exhibiting slightly higher values at

2.07 and 2.14 ds m<sup>-1</sup> (plots 4 and 12). Maize is not especially salt-tolerant, but these values did not seem to impact yields from those plots.

Average pH and EC readings by treatment are recorded in table 4.5. The dead termite mound soil treatment, on average, was planted in soils which were slightly more alkaline and slightly less saline than the other three treatments, with low standard deviation.

Table 4.5 Field pH and EC Values Averaged by Treatment.

_	Average pH	Standard Deviation	Average EC (ds m <sup>-1</sup> )	Standard Deviation
Treatment				
Control	5.37	1.71	0.94	1.04
Live Termite Soil	5.87	1.10	0.67	0.71
Dead Termite Soil	6.40	0.40	0.27	0.09
Manure	5.57	1.80	0.82	1.08

## 4.3.2. Yield

Yield metrics are displayed in table 4.6 below, including yield values which have been extrapolated to kg ha<sup>-1</sup> using the field plot yield data. These extrapolated values were used to compare yields from this study to observed yields from surrounding farms.

Table 4.6 Field Trial Yield Results.

	Total				Extrapolated
	Grain	Total	Grain	Extrapolated	Biomass
	Yield	<b>Biomass</b>	per Cob	Grain Yield	Yield
	(kg 0.0035 ha <sup>-1</sup> )	(kg 0.0035 ha <sup>-1</sup> )	(g)	(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )
Control	5.26	6.83	108.97	1,502	1,951
Living Termite Soil	3.93	7.69	86.66	1,123	2,197
Dead Termite Soil	5.26	7.84	98.68	1,503	2,240
Cattle Manure	6.01	8.65	113.94	1,715	2,471

The control yields observed in this study are higher than the local averages, which are around 350 kg ha<sup>-1</sup> per growing season. This is likely because of several different conservation agriculture methods used in this study which differ from local practices - use of mulch (cut grass) applied to the soil surface, conservation tillage, and planting in rows. Additionally, instead of being rain-fed, this field was irrigated using a gravity fed, well-water irrigation system. Another area of the farm where the study takes place grew test plots which compared common bean (*Phaseolus vulgaris*) yields using conservation tillage, planting in rows, mulch, and organic amendments (cattle manure) to traditional local farming. All of these test plots were grown under rain-fed irrigation. Common bean yields under traditional farming practices (no mulch, tilled, not planted in rows) were around 0.5 kg for a field 50 m<sup>2</sup> in area. Bean yields under sustainable farming practices were around 6 kg under the same planting area. Even without the addition of irrigation, rain fed fields can still be improved using simple sustainable farming techniques.

A storm with heavy wind caused damage to the field a few weeks before harvest. Secondary termites, rats, and moles contributed to some biomass and grain loss from the stalks which had blown over. The termites hollowed out the cobs which fell to the ground, but left the grain untouched, still edible and harvestable. This pithy cob material may be of special use to the termites as it is relatively resistant to further breakdown, and could provide benefit within the mound for building structures. Some cobs, stalks, and exposed roots were eaten by the rodents. Maize typically has a grain to biomass ratio of 1:1, which was not observed in this study. There were distinct differences between grain and biomass, with less grain being harvested for every single plot in the study. This is likely due to yield loss from rodents in lodged stalks.

Substantial lodging was observed in block 3, with less than 20 % of maize plants left standing at time of harvest in every plot. The living termite mound soil treatment ended up with 89 % of maize fallen over, the greatest of all plots in block 3. Block 2 had substantially less lodging, but the living termite mound soil treatment still experienced greater than 80 % lodging within the plot, whereas the manure treatment only had 42 % of stalks fallen over. Block 1 had the least amount of lodging overall, with percent lodged ranged from 44 % - 64 %.

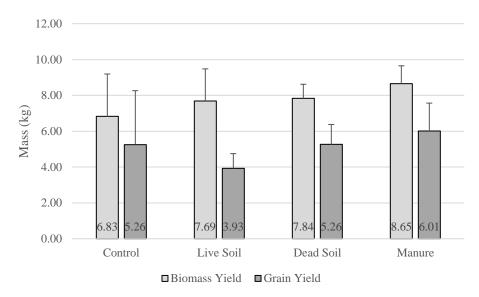


Figure 4.2 Total maize grain (kg) and biomass (kg) yield observed per treatment. Grain yield includes field-dry kernels which had been ginned. Biomass yield includes empty husks and stalk tissue. Standard deviation bars are included to represent deviation between treatments.

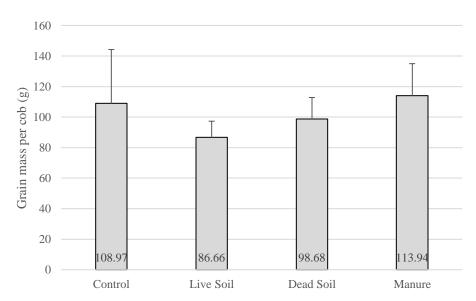


Figure 4.3 This figure displays dry grain mass (g) per cob post-ginning, averaged by treatment. These values represent field-dry maize kernels which were removed from the cob before being weighed. Standard deviation bars are included to represent deviation between treatments.

When considering total grain harvested from each treatment, statistical differences were observed at  $\alpha$ =0.1. Plots treated with cattle manure had significantly greater yields than all other treatments. Plots treated with dead termite mound soil, living termite mound soil, and no amendment (control) weren't statistically different from each other. Overall, the only treatments statistically different from each other were manure and living termite mound soil at  $\alpha$ =0.1. These differences were represented visually in figure 4.2.

Statistical differences in biomass yield were not observed among treatments, with P=0.6072 at  $\alpha=0.1$ . There was experimental error associated with this data collection from a variety of sources, including storm damage, termite damage, and rodent damage. Other factors such as field variability, the presence of an abandoned subterranean mound, and the selection of mounds used as amendment could also influence the results. To collect this data, stalks were bundled and carried a quarter mile to be weighed on a hanging kilogram scale. There was some yield loss associated with transport which was too time consuming to recover.

Statistical differences were observed at  $\alpha$ =0.1 when comparing average grain per cob recorded for different treatments, which is represented by figure 4.3. Maize cobs harvested from plants which were amended with cattle manure had significantly more grain than cobs from the dead termite mound soil treatment and the control, with Pr>F=0.0762 and R<sup>2</sup>=0.557. This was expected due to the higher plant nutrient concentration found in cattle manure compared to the dead termite mound soil and the control, which has no plant nutrients added. According to the Fishers LSD test, cobs

harvested from the manure treatment were not statistically different from the cobs harvested from maize plants which had been treated with living termite mound soil at  $\alpha$ =0.1. Grain per cob recorded for dead termite mound soil and control treatments were not statistically different from each other. Living termite mound soil treatment yields were not statistically different from either group. This result could be due to the greater amount of N applied in the living termite mound soil compared to the dead termite mound soil. The living termite mound soil used as amendment in this study had a concentration of 11.2 mg kg<sup>-1</sup> N, which is over twice as that of the dead termite mound soil (4.4 mg kg<sup>-1</sup>) used. Had the fertility data been available at time of planting, different mounds would have been selected which might produce different results.

When the mounds were selected for amendment, they were chosen based on proximity to the field trial rather than any other indicator. Fertility data was not received until March 2019, 7 months following the initiation of the study. If the fertility content of the mounds was known at the initiation of the field trial, perhaps different mounds would have been chosen to add higher concentrations of nutrients to the field. The fertility results showed that other mounds which were farther away from the field trial were moderately more fertile and would have been better suited for use as a fertility amendment than the ones actually used in the study.

In this case, even a non-significant yield increase should be noted because at the hectare scale, there was a 400 kg difference in yield between living and dead termite mound soil per growing season looking at the extrapolated yield data. Living termite mound soil, at the hectare scale, yielded around 400 kg less maize grain than the control,

which had no amendment added. While non-significant yield differences are not scientifically important, they are extremely important in these types of subsistence systems where any increase is a cause for celebration. In addition, non-significant results which still produce sizeable differences indicate potential for further improvement to the methodology. Selecting different mounds using recommended indicators from this study could provide higher concentrations of NPK as fertilizer and potentially increase yields at a significant level.

Something important to note is that a family of 6 needs 1,250 kg yr<sup>-1</sup> of maize grain to achieve food security if completely reliant on maize. This number drops down to around 750 kg yr<sup>-1</sup> when supplemented with other food sources. Farmers in the area surrounding the study site are happy to harvest 350 kg ha<sup>-1</sup> per growing season, for a total of 700 kg ha<sup>-1</sup> annually. When actual farm size is taken into account, which is often less than a hectare, the numbers drop to less than half that. The extrapolated yields surpass the value necessary to sustain a family of 6, but this should be attributed to the use of irrigation, mulch, and reduced-till management. While mulching and reduction of tillage are more easily accessible methods of field management, irrigation remains a challenge for a majority of farmers. The irrigation system in this study used well water which ran downhill through a series of plastic pipes and rubber tubing with small holes poked through at each planting hole. As both public and private sector construction projects increase in the area surrounding the study site, wells are being constructed in greater density. These wells are mostly available for public use, so irrigation (in varying

capacity) is becoming increasingly more accessible to subsistence farmers of Mayuge district.

Harvested roots were also examined for differences in root structure. Clear differences in lateral branching, brace root abundance, and fine root abundance were observed between treatments, displayed in figure 4.4.

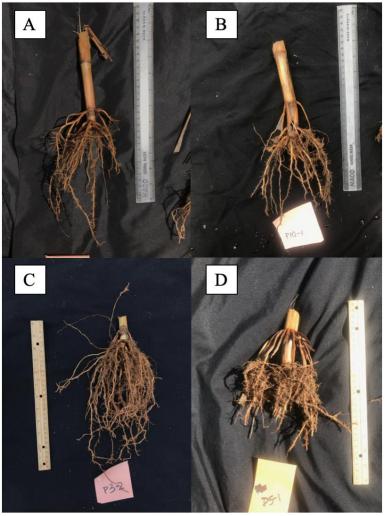


Figure 4.4 Examples of root architecture resulting from different amendment added. (A) Control, (B) live termite mound soil, (C) dead termite mound soil, (D) manure. The roots were photographed at the study site by Kimberlyn M. Pace.

Maize roots harvested from plots containing dead termite mound soil had greater density of fine roots and fewer brace roots than maize roots harvested from plots treated with manure. Roots treated with manure had a comparable density of fine roots compared to those grown with dead termite mound soil. Roots harvested from living termite mound soil did not develop as many fine roots as manure and dead termite mound soil treatments, and overall had less dense root systems, comparable to the control treatment with no amendment added. An increase in fine roots indicates greater soil-root contact, allowing the plant to explore greater volumes of soil for scarce resources. Nodal distance between brace roots and lateral roots was greatest in maize treated with manure or live termite mound soil, and smallest in maize treated with dead termite mound soil or no amendment.

Root structure relays information about local nutrient availability and the ability of maize plants to acquire nutrients, especially in degraded soils. Nutrient poor soils may produce plants with an increase in fine roots, which are useful for scavenging for water and phosphorus by increasing soil-plant contact points.

While maize treated with living termite mound soil had comparable biomass yields to other treatments, grain yields were smaller (figure 4.2). While this may be attributed to yield losses from lodging, it is shown here that the grain per cob is also less than that of other treatments (figure 4.3). Something within the living soil could be interfering with cob development through inhibiting root development, therefore limiting plant access to less mobile plant nutrients necessary for physiological development.

# 4.3.3. Arbuscular Mycorrhizal Fungi

The ability of maize roots to form mature associations with local mycorrhizae is extremely important in low input, degraded systems. Out of the entire study, these results were the most statistically significant in terms of benefit supplied by termite mound soil. Table 4.7 displays average AMF %, average rank, average arbuscule and average vesicle counts per root segment. Average vesicle and arbuscule counts by treatment are also displayed in figure 4.5 with standard deviation included. Average AMF % by treatment is displayed in figure 4.6.

**Table 4.7 AMF Field Trial Results.** 

	Average				
	Average	Average	Arbuscule	Average Vesicle	
_	AMF %	Rank	Count	Count	
Amendment					
Control	39.21	1.96	0.76	0.51	
Live Soil	30.21	1.54	0.54	0.27	
Dead Soil	48.13	2.43	1.23	1.32	
Cattle					
Manure	41.91	2.10	0.67	0.45	

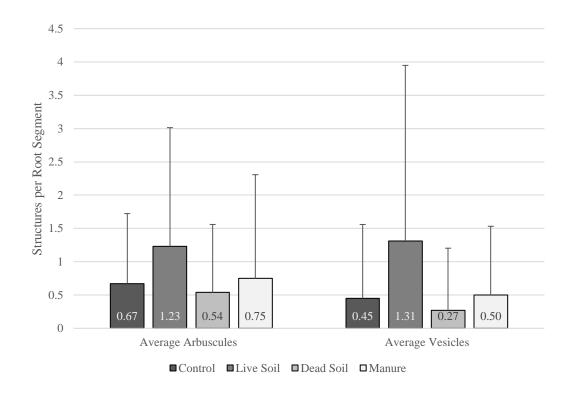


Figure 4.5 Average arbuscules and average vesicles observed per treatment. Structures were observed under 10x magnification. Standard deviation bars are included to represent deviation between treatments.

Maize plants which had been amended with dead termite mound soil had a significantly higher number of vesicles than live soil, significant at P=0.0342 at  $\alpha$ =0.05. Average vesicles found on maize roots treated with dead termite mound soil differed significantly from the control, maize roots treated with manure, and maize roots treated with living soil at  $\alpha$ =0.05. Maize roots treated with soil from a living termite mound had on average 0.27 vesicles per root segment, which was the lowest recorded value among all 4 treatments.

Maize plants treated with dead termite mound soil had significantly higher average arbuscules observed, significant at P=0.0012. Average arbuscules observed on maize roots treated with dead termite mound soil differed significantly from the control, maize roots treated with manure, and maize roots treated with living soil at  $\alpha$ =0.05. This value was significantly higher than the other three treatments, which were not statistically different from each other.

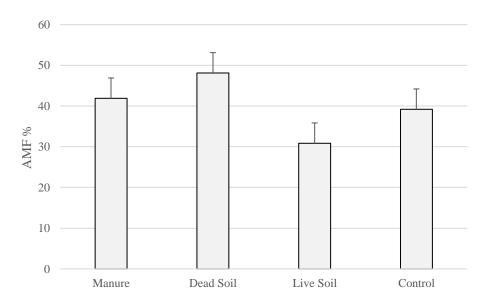


Figure 4.6 Average AMF percent by treatment, calculated using (Eq. 2). Standard deviation bars are included to represent deviation between treatments.

Significant differences were also observed between treatments when considering average rank. Root segments from maize plants treated with dead termite mound soil had an average rank of 2.4, which was statistically greater than average rank observed on maize plants treated with living termite mound soil, significant at P=0.0316. The living

termite mound soil treatment did not statistically differ from the control or the manure treatment. At  $\alpha$ =0.05, roots treated with dead termite mound soil were not statistically different from those treated with manure, or the control, but were statistically different from maize treated with living termite mound soil.

After AMF percent was calculated (Eq. 2), differences were observed between roots treated with dead termite mound soil and living termite mound soil, significant at P=0.0373. These differences can be seen in figure 4.6. At  $\alpha=0.05$ , roots treated with dead termite mound soil were not statistically different from those treated with manure, or the control, but were statistically different from maize treated with living termite mound soil.

The interactions observed in the study are consistent with literature citing antifungal properties of the *Macrotermes* mounds. There seems to be an inhibitory effect in applying living termite mound soil, because AMF percent of maize roots decreased from 48.91 % when dead termite mound soil was applied to 30.21 % when living soil was applied. Perhaps the antifungal property present in living termite mound soil has sufficiently degraded over an unknown number of years to the point where, once the mound has been abandoned for x amount of time, the protein or microbial aspect has been degraded. It is unclear how long this may take.

These differences may also be due to differing root architecture observed between treatments (figure 4.4). Maize treated with dead termite mound soil had increased density of fine roots, which grew to greater depth than living termite mound soil. Perhaps this decrease in fine roots, where AMF tend to colonize in greater density,

is an additional explanation for the significant decrease in arbuscules, vesicles, and overall infection density observed in the study. Roots treated with dead termite mound soil also tended to grow deeper into the soil profile, whereas roots treated with living termite mound soil tended to root shallower. Shallower roots are more likely to dry out in the soil closest to the surface between watering events, which is more unfavorable than the wetter, cooler soil located below. The soil from the living mound may have an additional inhibitory effect on root growth of maize, and as a consequence decrease the ability of AMF to infect roots, on top of the proposed anti-fungal properties of the termite soil.

AMF % is an important diagnostic criterion to assess the success of a soil treatment, especially in systems where crops rely on AMF infection to increase root surface area and scavenging ability.

Applying dead termite mound soil significantly increased AMF colonization of maize roots, indicating its usefulness as an agricultural amendment. Maize treated with dead termite mound soil exceeded manure in terms of AMF colonization especially when it came to prevalence of mature structures. In low input farming systems, this soil can almost be thought of as an AMF inoculant which is extremely beneficial for nutrient and water acquisition. While the inhibitory effect of living termite mound soil could also potentially decrease likelihood of soil pathogens and nematodes as it did AMF colonization, it is not worth sacrificing the roots ability to form these mycorrhizal associations. Additionally, the disease resistance offered by AMF associations is well documented throughout the literature.

While manure is often credited with the stimulation of soil microorganisms, this study revealed an additional stimulatory effect on AMF of applying soil from a dead *Macrotermes* mound, which may also be due to positive effects of dead termite soil on fine root growth (figure 4.4). Additional data on the composition of the remaining soil microorganism populations is not available for this study, but the increase in AMF density could be used to infer that there might be increased populations of other microorganisms as well, perhaps greater than that of manure. Keya (1982) recorded increased populations of bacteria, fungi, protozoa, and actinomycetes in dead mounds versus living mounds. This indicates that the anti-microbial component that the termites used has degraded or otherwise been destroyed over time, allowing populations to increase following the abandonment of the mound. Various species of brushy plants, grasses, and small trees growing on the mounds could act as potential hosts for AMF to persist on.

## **4.3.4.** Implications for Farmers

Manure seems to be the best option when considering which organic amendment to add in terms of fertility added, which was expected. The argument is not that termite mound soil will out-perform manure, but instead hopefully serve as a bridge solution to help farmers begin restoring their degraded soil while also slowly beginning to incorporate other sustainable farming techniques as they are able to afford them. It is a kick start to the restoration process, to give farmers a more affordable option to replenish soil health and boost crop production. In this case, some amendment is better than none,

and if the mounds are already being knocked down, it is important to investigate the potential benefit that this "waste product" could provide.

Using this data, 5 years fallow could be a recommended treatment for the restoration of fertility. This is in line with historic agricultural patterns in the region, where crop land was used until nutrients and other soil resources were depleted and then left to replenish as farmers moved on to uncultivated land. However, most subsistence farmers cannot afford to leave their field fallow for multiple growing seasons, so this management strategy could not be implemented without government or NGO assistance in the form of grain rations and payouts for remaining fallow.

Repeating this study on land which has been continuously cultivated would be beneficial because it would relay information about how the different amendments impact crop yields on land which is being actively "mined" by maize and other nutrient hungry crops. Additionally, a multi-year study could provide more information about the concentration of nutrients removed by the crop per growing season compared to how much is being supplemented by the various amendments. Decreases in soil and grain N and P might have been observed over time following repeated crops of maize.

It is unclear whether or not *Macrotermes* mound soil can provide a significant source of plant available P. Further testing will need to be done in order to accurately assess the distribution of the P pools in the area compared to those found in *Macrotermes* mounds. Because the fertility added is significantly less than that of manure, *Macrotermes* mound soil should not be championed as a replacement fertilizer equivalent to manure. However, these mounds are essentially above ground soil fertility

hubs, and so using excess soil can replace some fertility lost to agriculture. At any rate, any addition of fertilizer is better than none in low input systems, and when no other options are available, *Macrotermes* mound soil could be considered as a fertility supplement to degraded soils in the area.

The goal is to reverse some of the degradation that these soils are undergoing, and in turn increase the short-term fertility and economic value of these farms. This is envisioned as a first step in the slow climb to long term sustainability, which includes the integration of animals and adoption of additional best management practices (BMP)s by farmers (Waha, 2018).

### 4.4. Conclusions

Use of termite mound soil could primarily be thought of as an 'AMF inoculant' due to the importance of mycorrhizas in smallholder agriculture, and the stimulatory effect of applying dead termite mound soil to a crop of maize. The AMF data produced results with the highest level of significance, therefore it can be thought of as the primary benefit of applying mound soil to maize in this study. Had this been a multi-year trial on land which had not been fallow for 5 years, yield differences stemming from differences in AMF colonization and K added as fertilizer might have also been observed.

Many farmers hesitate to invest organic resources into their fields due to scarcity or physical difficulty. Dead *Macrotermes* mound soil is a viable amendment for smallholder farmers in Eastern Uganda and can successfully serve as a bridge solution to

encourage sustainability and agricultural self-sufficiency in other ways which benefit the farmer. Successful incorporation of one organic resource may lead to increased application of other resources such as manures and biomass after yield differences are observed.

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#### 5. CONCLUSIONS

Combining several sustainable practices can result in increased access to NPK without requiring investment in additional, expensive resources. These practices include the application of 350 ml soil from a dead termite mound to each planting hole, reducing tillage, and crop rotation between maize and a common legume already grown widely throughout the region (*Phaseolus vulgaris*).

The use of soil from a dead termite mound can be considered a K replacement for leaving a field fallow for 5 years, because the levels are comparable, even slightly higher in some cases. In addition, not only did dead termite mound soil allow for healthy AMF colonization compared to soil taken from a living mound, it actually stimulated it, which increases P acquisition. Reducing tillage will help with the retention of organic carbon and the stimulation of soil microorganisms, including AMF, biological N fixers, and decomposing organisms which will aid in the mineralization of organic nutrient sources. Crop rotation with a legume (*Phaseolus vulgaris*) is necessary in maize based cropping systems because of the additions of plant available N to the soil by *Rhizobium* bacteria, which is taken up by maize in subsequent growing seasons.

Using survey data from this study, there are 3.8 mounds per ha in this specific part of Eastern Uganda, and 1.24 living mounds per hectare, which each regenerate at a rate of 1 m<sup>3</sup> per mound per year (Pomeroy 1976b). Scaled up, there are 984 mounds per square mile, with 321 of those mounds being actively growing. Annual amendment replaced using yearly regenerated growth can be calculated easily by multiplying the

number of living mounds by 1 m<sup>3</sup>, therefore it is going to be assumed that at this study site, there is 1.24 m<sup>3</sup> amendment being created per year, with the assumption that 321 m<sup>3</sup> is being regenerated annually per square mile. As long as farmers use less than this total regenerated amount per year, this amendment can be used sustainably.

There are 536 people per square mile at the study site (Trading Economics, 2018 Rural Uganda Statistics). Based on the sustainable rate of harvesting the mounds (using only yearly regenerated growth), if there are 1.24 living mounds per ha, 536 people can use up to 321 m³ amendment per year for 1 square mile of land. Assuming a family size of 8, this works out to an estimated 100 farms per square mile, which can each use 3.21 m³ mound soil per year. At the recommended rate, 350 ml per planting station, and an assumed farm size of 0.1 ha, each farm can add termite soil to their entire field per year, for two growing seasons, with some left over. To fertilize a 0.1 ha field using the application method described in this study, 1.39 m³ amendment will be required per growing season, and 2.78 m³ will be needed annually. With 60 cm planting hole spacing, there will be 2,828 planting stations dug in a 0.1 ha field.

If the results of this study can impact only 5,000 people living in a 10-square mile radius of the study area, then this potential solution will have substantial value. While these yield increases aren't necessarily comparable to those in the United States, any yield increase that can be encouraged in these farms is a cause for celebration and will mean a great deal to the economic and nutritional livelihoods of these farmers. To easily determine how much soil can be used as amendment in a given area, farmers should simply count the number of living mounds in the area. These mounds grow at a

rate of around 1 m³ per year, an easy number to remember. To use this amendment at a sustainable rate, they need to harvest less soil from dead mounds than is being regenerated in living mounds every year. For example, if there are 5 living mounds and 5 dead mounds in a given area, farmers can responsibly use 5 m³ of soil per year without negatively impacting termite populations. 1 m³ of soil is slightly less than 2 wheelbarrows full of soil, which is an easy management strategy to communicate, given that there is at least one wheelbarrow present in the community. Simpler management strategies will have higher rates of adoption and so recommendations need to be communicated in ways which are easy to understand and visualize.

The recommendation would therefore be to apply at the beginning of each growing season, and to add the full recommended rate to each planting hole. Any excess mound soil could also be applied to planting holes or spread on top of the field nearest to the mound.

If maize yields increase with the application of dead termite mound soil paired with other sustainable farming practices, a smaller area of farm land can be used to grow an equivalent amount of grain, freeing up a small portion of land for specialty crops.

After several growing seasons of applying amendment, farmers should be encouraged to plant specialty crops such as coffee, plantains, passion fruit, and papaya, which can be sold for extra revenue. This extra revenue can be used to invest in poultry, goats, or pigs which would increase access to manure and livestock products.

Future research opportunities include a more detailed analysis of phosphorus pools within the termite mound, as well as chemical information on other nutrients

which may be present. Additional spatial data on distribution of nutrients within mounds located in Eastern Uganda should be collected to increase accuracy of fertility concentration predictions. Fertility sequestered within mounds varies between climates, soil types, and ecosystems, so before any management recommendations can be made for a specific area, chemical testing must be done to investigate any fertility benefit. Lastly, there is substantial opportunity for these results to be communicated to rural farmers in Eastern Uganda, so that they can develop a management strategy for applying dead termite mound soil to their fields in an efficient and sustainable way.

In order to achieve long term food security, restoring the degraded soils of this region is a necessary first step. This is feasible through the continued investment of available resources in the most efficient way possible. There is a lot more work to be done in the coming years, and this study is laying the groundwork for and providing preliminary data supporting a potential solution that can alleviate the pressures of food insecurity for thousands of subsistence farmers located across Uganda.