Quantifying bioturbation by soil macroinvertebrates in a broad leaf African savanna

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DECLARATION

I, **Candice Lamb** declare that this thesis, which I hereby submit for the degree **M.Sc. Zoology** at the University of Pretoria, is of my own work and has not been previously submitted by me for a degree at the current or any other tertiary institution.

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QUANTIFYING BIOTURBATION BY SOIL MACROINVERTEBRATES IN A BROAD LEAF AFRICAN SAVANNA

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ABSTRACT

Soil macroinvertebrates, such as termites and ants, are considered ecosystem engineers that perform ecosystem processes which impact ecological function. Termites are important bioturbators that mix soil through building and foraging activities. Termite mounds influence the heterogeneity of savannas. Quantifying the volume changes of termite mounds is essential because it indicates their role as important bioturbators in savannas. However, ants are important predators of termites and may have a top-down effect on termite-mediated bioturbation. I used an evaluated photogrammetry technique to quantify the change in volume of termite mounds over time for two termite genera, *Macrotermes* and *Nitiditermes* in a broad leaf African savanna. To assess the extent of top-down control on termite-mediated bioturbation, I used large-scale suppression treatments to reduce the abundance of ants and termites and then measured the lateral and vertical movement of an optical tracer in the upper 30 cm soil layer. The photogrammetric estimated volumes and dry weight of termite mounds revealed that on average *Macrotermes* can add 11.17 kg. week^{-1} and *Nitiditermes* can add 72.50 g. week^{-1} to their mounds. The mean weight of soil added over a 40-week period to *Macrotermes* mounds was 446.61 kg and 2.92 kg to *Nitiditermes* mounds. Termite-mediated bioturbation was influenced by changes in either ant or termite abundance at certain depths and time frames. The suppression of ant abundance promoted termite-mediated bioturbation, and the suppression of termite abundance reduced bioturbation. Therefore, the amount of soil moved by termites to build their epigeal structures highlights them as important bioturbators in a savanna and ants have a potential top-down control on termite-mediated bioturbation.

Key words:

bioturbation, ecological function, ecosystem engineers, macroinvertebrates, optical tracer, photogrammetry, predation, savannas, termites and ants, termite mounds, top-down control, weight, volume estimations

LIST OF ABBREVIATIONS

ANOSIM	Analysis of similarity
CV	Coefficient of variation
CT	Computer tomography
3D	Three-dimensional
DSLR	Digital single lens reflex
DSM	Dense surface model
GLM	Generalised linear model
MDS	Multidimensional scaling
LiDAR	Light detection and ranging
OLRE	Observation-level-random-effect
OSL	Optical stimulated luminescence
RMS	Root mean squared
RMSE	Root mean squared error
SD	Standard deviation
SEM	Scanning electron microscopy
SfM	Structure-from-Motion
SOM	Soil organic matter
t_8	8-week time frame
t_{32}	32-week time frame

t_{40}	40-week time frame
UAV	Unmanned-arial-vehicle
UV	Ultra-violet
$\Delta V_{abs}{}^{-wk}$	Absolute rate of change in volume per week
$\Delta V_{abs8}{}^{-wk}$	Absolute rate of change in volume for the first 8 weeks
$\Delta V_{abs32}{}^{-wk}$	Absolute rate of change in volume for the next 32 weeks
$\Delta V_{rel}{}^{-wk}$	Relative rate of change in volume per week
$\Delta V_{rel8}{}^{-wk}$	Relate rate of change in volume for the first 8 weeks
$\Delta V_{rel32}{}^{-wk}$	Relative rate of change in volume for the next 32 weeks
$V_{d\%}$	Percentage of difference in V_w and V_p
V_8	Termite mound volume at week 8 of the experiment
V_f	Termite mound volume at week 40 of the experiment
V_i	Termite mound volume at week 0 of the experiment
V_p	Volume estimated by the photogrammetric technique
V_w	Water displacement volume
WRF	Wits Rural Facility

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DISCLAIMER

Chapter 2 and 3 of this thesis have been prepared as separate manuscripts for submission to the *Journal of Ecology*. Therefore, some overlap in content may occur throughout the thesis and the chapters have been written in the plural rather than the singular to secure publishable entities.

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CHAPTER 1: INTRODUCTION

BACKGROUND AND RATIONALE

Bioturbation is defined as the mixing or reworking of sediments and soils by living organisms (Meysman et al. 2006). Ecosystem processes such as water and nutrient cycling, soil formation and sediment transportation, litter decomposition, and nutrient availability are influenced by bioturbation (Jones et al. 2006, Ali et al. 2013). Burrowing mammals and many soil macroinvertebrates (i.e., invertebrates found in soil that are larger than 2 mm in size) perform bioturbation in ecosystems (Jouquet et al. 2006, 2011, Hagenah and Bennett 2013, Valentine et al. 2017). Soil macroinvertebrates play an integral role in regulating soil structure dynamics (Jouquet et al. 2011). Many ecological processes are controlled by soil structure, including gas exchange, root growth, habitat choice of other organisms, soil microbial biomass and activity, and water infiltration (Bottinelli et al. 2015, Cheik et al. 2019). Soil characteristics such as carbon and soil organic matter (SOM) content, susceptibility to erosion, and mineral nutrient availability can also be affected by bioturbation (Jouquet et al. 2006, Bottinelli et al. 2015). Examples of soil macroinvertebrates known for influencing soil structure include earthworms, ants, and termites (Jouquet et al. 2006, Bottinelli et al. 2015). These macroinvertebrates are considered ecosystem engineers with extensive influence in temperate and tropical ecosystems because of their large populations (Lavelle 1997, Jouquet et al. 2006). Ecosystem engineers are defined as organisms that alter the surrounding physical environment by regulating the availability of resources (Jones et al. 1994, 1997). Macroinvertebrates influence ecosystems via their activities including foraging, burrowing, and building which influence the surrounding physical environment by regulating soil structure, and ecological services and functions (Lavelle 1997, Lavelle et al. 2006, Jouquet et al. 2006, 2011).

Earthworms are well-known ecosystem engineers in temperate and tropical ecosystems that change structural properties of soil and regulate plant growth and SOM content (Lavelle 1997, Blouin et al. 2013). Soil structure is changed through creating burrows, incorporating plant litter, mixing nutrients and organic material, decomposing SOM, and ejecting subsurface/surface casts into the soil (Jouquet et al. 2006, Blouin et al. 2013). Burrows promote water and gas movement in the soil and SOM content is regulated by mixing organic material in earthworm casts (Jouquet et al. 2006). In Europe, on various land-use types, Feller et al. (2003) reported that earthworms bring ~ 40-ton ha^{-1}. year^{-1} of soil to the surface, which is indicative of their importance as soil ecosystem engineers in temperate regions.

In semi-arid savannas across the tropics, termites are prominent ecosystem engineers because of their ability to remain active in harsh environments or severe dry seasons (Jouquet et al. 2011). They construct epigeal structures that provide stable humidity and water retention which allows termites to avoid desiccation in harsh environments (Lavelle et al. 2006, Jouquet et al. 2011). Termites perform bioturbation by translocating soils horizontally and vertically from various depths in the soil profile to build these structures such as galleries, sheetings, and mounds (Sileshi et al. 2010, Jouquet et al. 2011). As termites bioturbate they alter soil physical properties by reducing bulk density and increasing water infiltration rates (Jouquet et al. 2011, Kaiser et al. 2017). While bioturbating, termites can also alter the chemical structure of soil through the inclusion of SOM, found within soil aggregates, into the soil profile (Bottinelli et al. 2015). Bioturbation causes the indirect input of SOM into the soil profile via the subsurface mixing of soil and the addition of plant litter into biogenic structures such as mounds and galleries (Rumpel and Kögel-Knabner 2011). Plant litter sequestration and waste disposal of dead tissues into nests, mounds, and galleries is a direct input of SOM into the soil profile (Rumpel and Kögel-Knabner 2011). The construction of galleries during activities plays a vital role in regulating nitrogen (N) mineralisation and nitrification, and nitrous oxide (N_2O) and carbon dioxide (CO_2) emissions (Ngugi and Brune 2012, Hu et al. 2017). Termites select for clay particles to build mounds and nests and alter the mineralogical properties of silicate clays to create clays that are expandable (Jouquet et al. 2002b, 2002a). Studies demonstrate that fungus-growing termite mound structures are enriched with finer clay particles compared to the surrounding landscape which modifies the pH and cation concentration of the soil (Holt and Lepage 2000, Jouquet et al. 2004, 2011).

Savannas are characterised as spatial heterogeneous ecosystems composed of a mosaic pattern of trees and grasses (Scholes and Archer 1997, Torello-Raventos et al. 2013). In the past, spatial heterogeneity causing the mosaic pattern was thought to be maintained by periodic fires and herbivory (Solbrig 1996). In recent years, there is increasing evidence that the presence and high biomass of soil macrofauna, like termites, also promotes spatial heterogeneity of the landscape in savanna ecosystems in addition to herbivory and fire (Sileshi et al. 2010, Joseph et al. 2013, 2014, Davies et al. 2016a). The soil on fungus-growing termite mounds is higher in SOM content than surrounding soils (Holt and Lepage 2000). The enrichment in SOM content creates nutrient hotspots that promotes the patchiness of vegetation growth characteristic of a savanna ecosystem (Jouquet et al. 2011, Joseph et al. 2013, Davies et al. 2016a). In Kruger National Park, South Africa, Davies et al. (2016b) found that termite mounds can enhance herbivory and influence up to 30% of the savanna landscape. Termites provide large-scale benefits in tropical ecosystems because they account for 40% to 65% of the soil macrofauna biomass (Wood 1988) and their mounds have high densities, averaging between 0.46 mounds ha^{-1} and 2 mounds ha^{-1} (Davies et al. 2014).

Quantifying the volume of soil added to termite mounds can indicate the magnitude of bioturbation performed by termites in a savanna. The typical approach to measuring the volume of termite mounds has been to estimate the overall shape of the mound using cone, spheroid, or cylinder geometrics (Josens and Soki 2010, Jamali et al. 2011, Tuma et al. 2019). This approach works well for simpler morphologies of termite mounds but less so for more complex morphologies with buttresses and multiple chimneys (Nauer et al. 2018). A more invasive approach is to dismantle the termite mound and use water displacement to measure the volume of the dismantled sections (Holt et al. 1980). However, to quantify temporal change in volume of termite mounds (growth or shrinkage), a less invasive and non-destructive approach, not limited by morphological complexity of mounds, is required. Three-dimensional (3D) photogrammetry uses a set of over-lapping photographs taken from multiple perspectives to obtain accurate information about an object (Lange and Perry 2020). Structure-from-Motion (SfM) software detects and matches common features between the set of photographs and generates a 3D dense surface model (DSM) of the object (Luhmann et al. 2020). Photogrammetry has been used in a wide range of applications from accurately estimating the growth of corals (Ferrari et al. 2017) to modelling extremely small objects like dental morphology (Silvester and Hillson 2020). Nauer et al. (2018) previously used and recommended photogrammetry to estimate the volume and surface area of termite mounds to determine methane flux measurements. Therefore, using photogrammetry to estimate the rate of change in volume of termite mounds, allows us to evaluate the utility of photogrammetry in bioturbation studies and provides insight into important processes influencing ecosystem function.

Ants are also considered important ecosystem engineers because of the various roles they have in ecosystems as omnivores, seed harvesters and dispersers, and predators (Christianini and Oliveira 2010, Del Toro et al. 2012). Ants construct nests that have large volumes near the surface of the soil, where most of their bioturbation activity takes place (Tschinkel 2004). Ants contribute to the return and cycling of SOM content through extensive mixing of the soil surface (Mora et al. 2005, Del Toro et al. 2012). Ants impact sediment loss and water infiltration through the presence of large macropores constructed during nest building (de Bruyn and Conacher 1990). Sediment brought to the surface by ants is susceptible to erosion (Cerdà and Jurgensen 2008). In eastern Spain, Cerdà and Jurgensen (2008) found that ants influence water infiltration rates, soil bulk density, and erosion after heavy rainfall events on over-managed orange orchards. However, the effect ant bioturbation has on surrounding environments is largely dependent on the species and its respective nest building characteristics (Frouz and Jilková 2008). It has been suggested that in comparison with other important bioturbators, such as termites and earthworms, ants can have comparable or larger surface soil deposition (Richards 2009, Richards et al. 2011, Taylor et al. 2019). In south-eastern Sweden coniferous forest, Taylor et al. (2019) estimated that ant bioturbation performed by *Myrmica* spp. and *Lasius niger* was 450 kg ha^{-1}. y^{-1} (dry weight).

In tropical and subtropical regions, ants and termites consist of a sizeable portion of the biomass with high abundance and species richness resulting in frequent encounters between the two macroinvertebrate taxa (Dial et al. 2006). The predation of termites by ants is an important ecological interaction between these two macroinvertebrate taxa (Yusuf et al. 2014, Frank and Linsenmair 2017, Walker et al. 2022). Therefore, the removal of ants could potentially cause a top-down effect on ecosystem processes (Parr et al. 2016, Walker et al. 2022). On the same study site utilised in this thesis, the suppression of ant abundance resulted in a top-down effect on herbivory (Parr et al. 2016) and decomposition (Parr et al. 2016, Walker et al. 2022), another important ecosystem process. Therefore, ant suppression is one way to quantify their influence on termite-mediated bioturbation. The importance of ants and termites in structuring ecosystems from below the surface via bioturbation is apparent and it is essential to quantify the effect the interaction between these two macroinvertebrate taxa have on bioturbation. To determine the effect that termites and ants have on the movement of soil, I used a large-scale suppression experiment to reduce ant or termite abundances and then observed the movement of an optical tracer through the top 30 cm of the soil profile. Various studies have attempted to assess the rate of bioturbation in landscape models using luminescence (Stockmann et al. 2013, Johnson et al. 2014) or optical tracers in marine ecosystems (Solan et al. 2004). Stockmann et al. (2013) and Johnson et al. (2014) used optical stimulated luminescence (OSL), a method that dates sediment or mineral grains by measuring the time elapsed since the grains were last exposed to sunlight during transport and prior to deposition. In Sweden, Solan et al. (2004), used natural sediment treated with dye that fluoresces under ultra-violet (UV) light and a sediment profile imaging camera optimised for fluorescence detection to observe the movement of sediment over a 16-hour time frame. My study represents the first to use an optical tracer to observe lateral and vertical bioturbation in the soil profile.

OBJECTIVES AND THESIS STRUCTURE

The objective of the studies conducted for this MSc thesis were to quantify the movement of soils by ants and termites in an African savanna as it is important in understanding their role in soil formation and the ecological processes effected by bioturbation performed by them in the ecosystem.

This thesis contains two data chapters that each make use of a different non-invasive technique to quantify the importance of ants and termites in bioturbation in a savanna. Each chapter is laid out in the format of a publishable article, and they are followed by a general discussion that examines the main findings of the research and its significance.

The first data chapter (Chapter 2) aims to quantify the significance of termites as bioturbators in a broad leaf African savanna. To achieve this, a photogrammetric technique that estimates the change in volume of termite mounds over time was developed and evaluated. The volume estimates were then used to quantify the amount of soil moved by termites to build mounds.

The second data chapter (Chapter 3) aims to quantify the downward and horizontal subsurface movement of soil in the savanna ecosystem and to quantify the role either soil macroinvertebrate taxa has in bioturbation in a savanna.

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CHAPTER 2:

USING PHOTOGRAMMETRY TO ASSESS THE VOLUME CHANGES OF TERMITE MOUNDS

ABSTRACT

Soil macroinvertebrates considered to be ecosystem engineers, such as termites, are essential in regulating the structure of soil which influences many ecological functions. Bioturbation by termites continually integrates and cycles important nutrients and minerals in subtropical ecosystems like savannas. Termite bioturbation is when soil is mixed and translocated through activities such as building epigeal mounds. These mounds have a strong influence on the heterogeneity of savanna landscapes. Quantifying the volume changes of termite mounds is important because it is indicative of their role as important bioturbators in a savanna landscape. We evaluated photogrammetry as a technique for quantifying the volume of termite mounds and then used this approach to assess the rate of change in volume of *Macrotermes* spp. and *Nitiditermes* spp. mounds over time in an African savanna. The chosen photogrammetric technique consistently overestimated volume but with high precision and low error values compared with previous studies. Our study focussed on the biological reasoning behind the change in mound volumes over time so the overestimated volumes could be regarded as reasonably accurate. Using the estimated volumes and mass of termite mounds, we found that on average *Macrotermes* can add 11.17 kg. week^{-1} and *Nitiditermes* can add 72.50 g. week^{-1} to their mounds. The mean weight of soil added over a 40-week period to *Macrotermes* mounds was 446.61 kg and to *Nitiditermes* mounds was 2.92 kg. This study is the first to use a Structure-from-Motion (SfM) photogrammetric technique to estimate the temporal change in volume of termite mound to quantify bioturbation. Our study further demonstrates how photogrammetry is a suitable technique to estimate the volume of termite mounds because of its quick, non-invasive field application. The quantified volume changes of termite mounds in our study highlights the importance of termites as bioturbators that promote spatial heterogeneity in a savanna ecosystem.

Key words:

bioturbation, ecosystem engineers, high precision, macroinvertebrates, *Macrotermes*, *Nitiditermes*, rate of change, savannas, SfM photogrammetry, termite mounds, volume estimations, weight of soil

INTRODUCTION

The presence of soil macroinvertebrates (i.e., invertebrates > 2 mm in size) in the soil profile are essential in regulating the dynamics of soil structure (Jouquet et al. 2011). The structure of soil - such as organic matter content, gas exchange, mineral nutrients, microbial activity and biomass, water infiltration, and susceptibility to erosion - influences many ecological functions (Bottinelli et al. 2015). Some macroinvertebrates have such a significant impact on soil structure and dynamics that they are considered to be soil ecosystem engineers (Jouquet et al. 2006, Bottinelli et al. 2015). Examples of soil ecosystem engineers that have an extensive effect on soil structure and dynamics are earthworms and termites (Jouquet et al. 2006, Bottinelli et al. 2015).

Termites are ecosystem engineers because they play a major role in ecological processes such as soil formation and regulation, decomposition, and nutrient cycling (Jouquet et al. 2011, Bonachela et al. 2015, Govorushko 2019). Important nutrients are continually added to and cycled in the environment through decomposition and the mixing of soil (i.e., bioturbation) that are performed by termites (Erpenbach et al. 2013, Dorn 2014, Jouquet et al. 2016). Earthworms have been described as one of the most prominent soil engineers involved in the same ecological processes in the temperate and tropical regions, while termites are a crucial soil engineer in subtropical savanna ecosystems (Jouquet et al. 2006).

In subtropical ecosystems, termites are notable ecosystem engineers because of their high abundance and population densities (Bignell and Eggleton 2000, Dahlsjö et al. 2014, Govorushko 2019). The ability of termites to remain active in harsh environments or severe dry seasons, because of the protection provided by their epigeal structures, allows them to dominate the decomposition process and the provisioning of other ecosystem processes in tropical and subtropical environments (Lavelle et al. 2006, Jouquet et al. 2011). This is especially important because other taxa, like dung beetles, that usually fill this ecological role become inactive during this time (Lavelle et al. 2006, Jouquet et al. 2011).

Most termite activity takes place below the soil surface during the construction of their subterranean galleries and nests, but above the soil surface, termite activity leads to the translocation of soil to build epigeal structures like soil sheetings and mounds (Holt and Lepage 2000, Jouquet et al. 2011, 2016). Sheetings are temporary soil structures, built by termites, that are placed over potential food items and the surface of the ground for protection (Jouquet et al. 2002a, 2011). When termites translocate and mix soil during foraging and building, they cause physical and chemical changes to the soil such as different hydraulic properties and nutrient concentrations (Li et al. 2017). Their activities can disrupt surface crusts, which reduces soil compaction. The creation of tunnels for foraging increases soil porosity which improves water infiltration and retention in the soil (Kaiser et al. 2017). This reduces erosion because there is less run-off (Léonard and Rajot 2001). Soil is translocated from deeper in the soil profile for the construction of mounds. In a dry deciduous forest in southern India, Jouquet et al. (2017) found that *Odontermes obesus* uses soils from the ~ 70 – 120 cm soil layer to construct their mounds. Soil profile formation can be affected by termites as they select for finer grained soil (e.g., clay; < 1 – 2 mm) to build their mounds which differs from the coarser soil grains found in the surrounding landscape (Jouquet et al. 2005, 2017). The higher clay content chosen by termites is for strengthening the cohesion between soil aggregates to reinforce their mounds for protection against predators and regulation of their environment (Jouquet et al. 2002b, Oberst et al. 2016).

Sheeting acts to protect termites from desiccation and predation (Jouquet et al. 2002a, Kaiser et al. 2017) and has been suggested to help support the weight of plants that are being consumed by termites to avoid potential collapse (Oberst et al. 2016). It has been argued that soil sheetings constructed by fungus-growing termites in Africa consist of soil translocated from the uppermost (0 – 3 cm) surface layer (Diouf et al. 2006, Villenave et al. 2009). However, in southern India in Vertisol, Cheik et al. (2019) found that carbon (C) content in sheetings suggests that soil translocated from below a 30 cm depth is used for their creation. This demonstrates that termite foraging activities can be further associated with changes in the C content of soil because old organic matter is translocated to the surface with soil from deeper than 3 cm (Cheik et al. 2019). Decomposition performed by termites also contributes to C and nitrogen (N) cycling in the soil as it is enriched in C and N through decomposing matter and subsequently mixed and translocated into C and N impoverished soil (Yamada et al. 2006, Neupane et al. 2015). The addition of organic matter into the soil also promotes microbial activity which causes the enrichment in cations (e.g., Na^+, K^+, Mg^{2+} and Ca^{2+}) and nutrients (e.g., NO_3^- and NH_4^+; Jouquet et al. 2004). Consequently, soil fertility and quality are enhanced, which is especially important for ecosystems such as savannas known to have leached soils (Jouquet et al. 2004, Major et al. 2012). The biomass of termite bodies and their faeces also enriches the soil with organic matter and can promote soil fertility (Jouquet et al. 2011).

Termite species in the sub-family Macrotermitinae are conspicuous and highly influential in African savannas (Dangerfield et al. 1998). Prominent features of African savannas are the large epigeal mounds that are created by termite species in the genus *Macrotermes* that promote heterogeneity, especially in nutrient-poor geologies (Dangerfield et al. 1998, Davies et al. 2014a, Muvengwi et al. 2017). In Kruger National Park, South Africa, Davies et al. (2014a) found that termite mounds are clustered throughout the landscape with density averaging 0.46 mounds ha^{-1} and up to 2 mounds ha^{-1} on landscape crests. These epigeal mounds influence the heterogeneity of vegetation patterns in savannas by being the location of nutrient hotspots that can support a greater number of forb and woody plant species, as opposed to the surrounding savanna (Jones et al. 1997, Moe et al. 2009, Erpenbach et al. 2013, Howison et al. 2016). They are also said to be focal points of tree regeneration in savannas (Moe et al. 2009). Additionally, the higher nutrient content on termite mounds promotes the growth of grasses that have a higher nutritional value for herbivory and mounds can influence up to 30% of the landscape as keystone structures that influence savanna heterogeneity (Davies et al. 2016b).

In savanna ecosystems the estimated biomass of termites is 70 – 110 kg. h^{-1} (Govorushko 2019). The construction of epigeal structures like mounds and sheetings can result in the bioturbation of between 3- to- 53- ton ha^{-1}. y^{-1} of soil in temperate regions and 730- to- 1100- ton ha^{-1}. y^{-1} in tropical regions (reviewed in Wilkinson et al. 2009). Bioturbation conducted by termites when building their mounds can be especially significant because as much as 190 kg of wet soil can be added over one rainy season to one termite mound (Turner et al. 2006). Surface soil deposition by burrowing invertebrates has been previously quantified using indirect estimates (reviewed in Wilkinson et al. 2009 and Bétard

2020). Some examples include calculating soil egestion rates and total biomass for earthworms and multiplying the number of ant nests per hectare by their dry weight and then dividing that by nest residence time (Richards et al. 2011, Taylor et al. 2019). In Namibia Turner et al. (2006) collected and weighed newly deposited soil on termite mounds in open grassland acacia savanna to quantity soil transported by termites.

Quantifying the volume of termite mounds for bioturbation is important because of the large volumes of soil that are translocated during their construction. The volume of soil added to a mound is indicative of the role that termites play as bioturbators in the savanna ecosystem. The external parameters of termite mounds, such as size/volume, have also been the basis for estimating regional termite abundance and populations (Darlington and Dransfield 1987, Jones et al. 2005, Josens and Soki 2010). However, the complex morphology and structure of termite mounds makes quantifying their volume challenging (Nauer et al. 2018). One approach to quantify the movement of soil related to mound building is with photogrammetry.

Photogrammetry is the science of obtaining accurate information about an object from photographs (Lange and Perry 2020). In three-dimensional (3D) photogrammetry, a series of overlapping photographs are taken of an object from multiple perspectives. Structure-from-Motion (SfM) software is then used to detect and match common features found in each of the over-lapping photographs to construct a digital, true-to-scale, 3D model of the object (Luhmann et al. 2020). It is a non-invasive technique that supplies a permanent 3D record of an object that can be measured at any time (McCarthy and Benjamin 2014). Photogrammetry has been used for many applications in the past such as monitoring the growth and volume change of coral, volcanic eruptions, and termite mounds (Diefenbach et al. 2012, Nauer et al. 2018, Lange and Perry 2020). In an Australian coastal savanna woodland, Nauer et al. (2018) successfully quantified external and internal termite mound parameters (e.g., volume, surface area, and porosity) using photogrammetry and X-ray computer tomography (CT) scanning to improve the accuracy of mound methane (CH_4) emission estimates. Therefore, the volume changes of termite mounds over time can be assessed using photogrammetry, which can provide insight into the volume of soil that is moved in the savanna ecosystem to build mounds and highlight the significance of termites as soil engineers.

The aims of this study were (1) to evaluate photogrammetry as a technique for quantifying termite mound volume, (2) to use this technique to quantify the changes in volume of termite mounds over time, and (3) to assess if there were seasonal changes in the volume of *Macrotermes* spp. mounds. We quantified the changes in termite mound volume by (a) observing how many mounds increased and decreased in volume over time, (b) if initial mound size affected the volume added to a mound (i.e., growth), (c) assessed the difference in the rate of volume of soil added to mounds between *Nitiditermes* spp. and *Macrotermes* spp., after accounting for initial mound size, and (d) calculated how much volume in a mound can be attributed to pore space. Our study investigates the importance of termites as bioturbators in a savanna ecosystem and is the first to use photogrammetry as a technique to estimate the change in volume of termite mounds over time for bioturbation purposes.

MATERIALS AND METHODS

Study site

The field component was conducted at Wits Rural Facility (WRF, 24.5667º S, 31.0985º E), South Africa. The WRF has an ~ 580 m elevation and is 350 ha of lowveld savanna with nutrient-poor sandy soils. The lowveld savanna is dominated by tree species including *Combretum collinum*, *Dichrostachys cinerea*, *Sclerocarya birrea*, and *Terminalia sericea* (Parr et al. 2016). The grass layer is predominantly *Heteropogon contortus*, *Hyparrhenia* spp., *Panicum maximum*, and *Pogonathria squarrosa* (Parr et al. 2016). WRF receives annual rainfall of 648 ± 194 mm (1992 – 2022) mostly during the summer months with a dry season between May and October.

To understand temporal changes in termite mound volume, termite activity was monitored by placing 16 toilet-roll baits per control plot (four control plots). The baits were scored depending on how much of the bait was consumed 0 – 25% (score 1); 25 – 49% (score 2); 50 – 75% (score 3); 75 – 94% (score 4); 95 – 100% (score 5). The baits were assessed and scored monthly. Weekly rainfall data (in mm) was recorded by WRF for the experimental period.

Camera calibration

Photographs were taken using a digital single-lens reflex (DSLR) camera (Canon EOS 700D) and a selected lens (Canon 18 – 55 mm STM). The DSLR camera and lens were calibrated for the photogrammetric process. Calibration corrects for radial and decentring lens distortion across the camera's field of view (Postma et al. 2013). Calibration was achieved by taking photographs at a set minimal and repeatable focal length (18 mm) and at multiple prescribed angles of a calibration grid of coded targets provided by PhotoModeler ® Premium (EOS Systems Inc). This focal length also provides a wider angle of view and low magnification that is suitable to photograph larger termite mounds (e.g., *Macrotermes* spp.). To reduce unnecessary calculations during processing, red-eye, auto rotation and image stabiliser settings were turned off for both calibration and field use (Postma et al. 2013). PhotoModeler ® calibration procedures are accurate and repeatable for close-range photogrammetry (Remondino and Fraser 2006).

Field experimental procedure

Thirty-three termite mounds of varying sizes and genera were selected in the field. Twenty mounds were identified as *Nitiditermes* spp. (previously known as *Cubitermes* spp.) and 13 as *Macrotermes* spp. mounds.

Before photographs were taken, mounds were visually assessed for possible biological and environmental damage. Damage to termite mounds can increase the risk of predation or disturb the colony resulting in the abandonment of the mound (Tuma et al. 2020). Termite mounds needed to be occupied by termites to be included in this study as this would influence the mound building process. Therefore, mounds that showed signs of abandonment were excluded from this study. None of the mounds were damaged via biological nor environmental disturbance during the study. Multiple photographs were taken of all 33 termite mounds in late November 2019 (Week 0) and in late September 2020 (Week 40). Multiple photographs were only taken of the 13 *Macrotermes* mounds in late January 2020 (Week 8).

Each mound (subject) was photographed (at least 40 photographs) from multiple angles per project, including angles close to 90° to capture its entire surface area. Consecutive photographs overlapped to ensure detectable points in the background and on the subject for photographic orientation of the subject in a 3D space in the PhotoModeler ® Premium software. Triangular markers and rulers were placed around the termite mounds (see supplementary material Fig. S1) to aid feature detection for photographic orientation and to scale the scenes. Before the photographs were taken, a 2 m radius of vegetation was cleared around the mounds to minimise obstruction and movement of vegetation between photographs. Movement of vegetation would sometimes prevent the software from orientating sets of photographs and a 3D model of the mound could not be generated.

We calculated the proportion of mound volumes attributed to pore space encompassing galleries and tunnels. Two *Nitiditermes* mounds were cut in cross-section near the base and removed from the field. The cross-section ensured that the soil below the ground plane was not accidentally included in the mound volume. Only *Nitiditermes* mounds could be used as *Macrotermes* mounds were too large for an effective cross-section to be performed. The surface where the cross-section took place was smoothed out to ensure it was flat. The cut-off portion of mounds were then placed on a flat, smooth surface to assist in accurate digital ground plane creation for the 3D modelling process. The same photogrammetric technique, as described above for the mounds in the field experiment, was then used to estimate the volume of these cut-off mounds. The mounds were then crushed to the same consistency in separate rigid containers where no soil could escape. The soil for each crushed mound was then measured using a beaker to get its volume. The pore space volume was calculated by subtracting the crushed volume (L) from the photogrammetric estimated volume (L). A portion of the soil (0.50 L) from each crushed mound was weighed and then oven dried at 105 °C for 48 hours to determine the water content. A previous technique to assess pore space was performed (see supplementary material) but that technique allowed for soil below the ground surface to be included in the volume of the mounds and could not be used. The density of mound soil (density = mass/volume) was calculated using the dry weight and photogrammetric volume estimation for each mound. Density was then used to calculate the minimum, maximum and average weight of soil moved by the two termite genera.

Accuracy and precision assessment

To evaluate the accuracy and precision of the photogrammetric process, three inverted metal bowls of differing sizes (small – 3.90 L, medium – 5.60 L, and large – 45.20 L) were used as artificial mounds for volume estimation (Fig. 1). Each bowl was painted with a water-resistant matte brown paint and spattered with yellow to reduce glare and provide a more heterogeneous texture to aid in automatic feature detection and matching.

Water displacement was used to measure the volume of each artificial mound (i.e., metal bowl) separately in litres. A known volume of water was placed in rigid containers (reservoirs) large enough to hold the artificial mounds. The artificial mounds were inverted and pushed down into the water ensuring that no water entered them. The water level was marked before and after the artificial mounds were placed into the reservoirs. They were then removed from the reservoir and water was added to the mark that was made after the artificial mounds were pushed down into the water. The volume of water added was recorded as the displacement volume of the artificial mound.

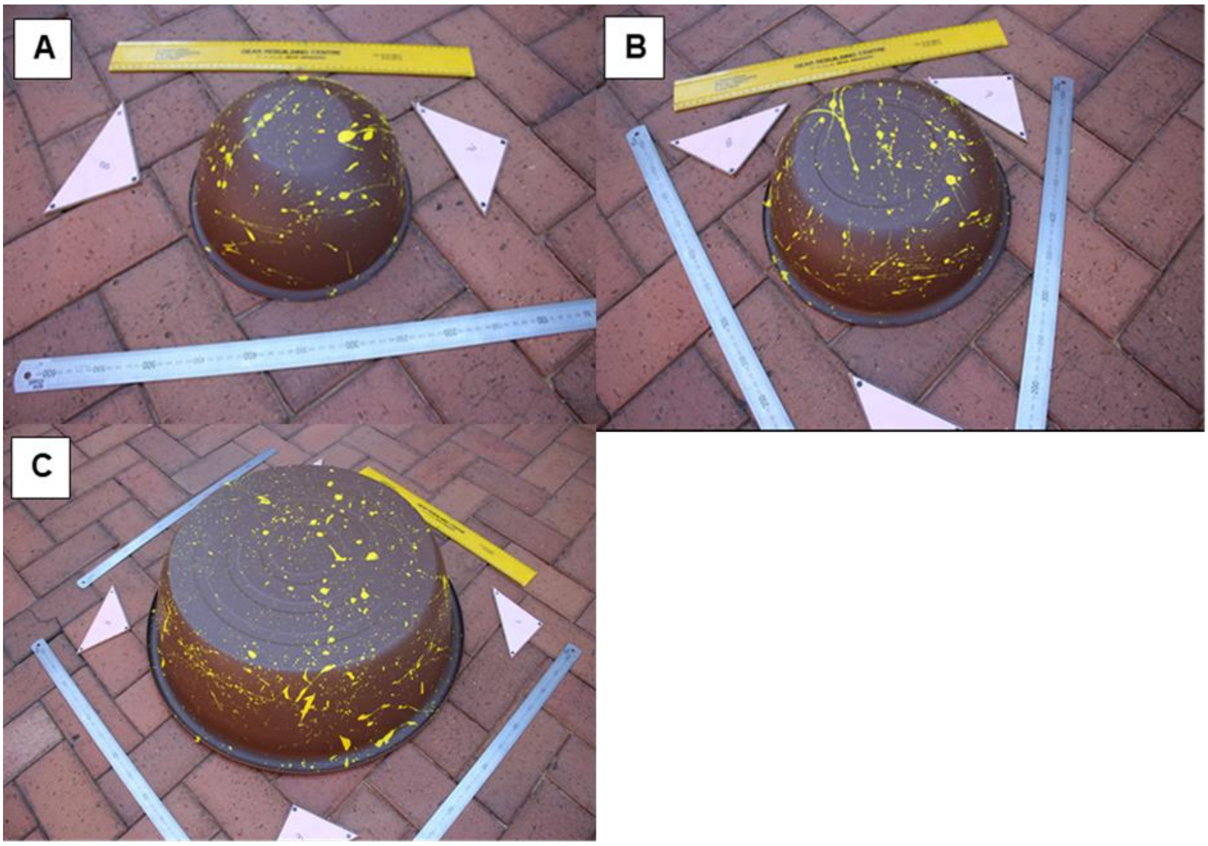

Figure 1: Images of **A)** small (3.90 L), **B)** medium (5.60 L), and **C)** large (45.20 L) metal mounds that were painted brown and spattered with yellow paint to create a matte heterogeneous surface. For scale, each metal ruler was 60 cm in length.

For validation purposes, five projects, containing at least 40 photographs each, were taken of each artificial mound for multiple rounds of photogrammetric processing to account for human error and to test for accuracy and precision. Each round was performed on a different day to account for temporal variation. As previously, triangular markers and rulers were placed around the subjects (Fig. 1). All artificial mounds, markers and rulers were placed on a flat, smooth surface to assist in accurate digital ground plane creation. In a pilot study, mounds of building sand were used to simulate termite mounds for the accuracy and precision assessment. The mound volume was calculated manually, but compaction of the sand was identified as a potential problem and this method could not be used (see supplementary material).

Photogrammetric volume estimation

PhotoModeler ® Premium version 6.3.3, a commercially available three-dimensional modelling package, was used to estimate volume of the subjects (de Bruyn et al. 2009). Three-dimensional spatial models of the metal and termite mounds were created from each set of projects using a SmartMatch ® (PhotoModeler ® Premium version 6.3.3) function and dense surface modelling. The SmartMatch ® function detects and matches common features between overlapped digital photographs in a project, marking them at the highest point density. Point cloud generation with triangulation (dense surface modelling) reconstructed the 3D surface of the well-photographed areas (see supplementary material Fig. S2). The feature detection in PhotoModeler ® Premium generated a distinct soil-termite mound boundary for the dense surface models (DSMs). During dense surface modelling, the immediate surface around the mound can also be modelled and will add to the volume of the mound if not removed. Therefore, any surface that did not form part of the mound was removed by cropping around the soil-termite mound boundary and deleting that part of the model. Surface textures were generated for each DSM, using the assigned photographs, to further verify which part of the DSM represented the mound versus the surrounding ground. A plane was then generated in the 3D space as the best fit for SmartPoints ® that represented the ground immediately around the mound. The plane seals the 3D model of the mound for volume estimation, where the mound is presumed to be solid. To designate real-life scale accuracy for the volume estimation, the appropriate distance was assigned between two points manually cross-referenced on a ruler (i.e., physically in the photographs as reference points with a measured distance) from at least 10 digital photographs of the project. Project accuracy was measured using the range of maximum residuals of the mounds (2.1 to 4.8 pixels – Root mean squared [RMS] error) against the recommended upper limit of 5 RMS (see PhotoModeler Scanner help file).

Statistical analyses

Accuracy and precision assessment

To evaluate whether the chosen photogrammetry technique was suitable for quantifying the volume of termite mounds, the percentage of the difference ($V_{d\%}$) between the volume of artificial mounds measured by water displacement (V_w; L) and the volume estimated by the photogrammetric technique (V_p; L) was calculated (Eq. 1).

$$V_{d\%} = \left(\frac{V_p - V_w}{V_w}\right) * 100 \qquad \text{Eq. 1}$$

The calculations were applied five times (per round) to each artificial termite mound. To estimate potential errors that were introduced into the modelling process, standard deviations (SDs) were calculated from the five rounds for each of the artificial mound sizes. Further, a coefficient of variation (CV; SD/mean * 100) was calculated for comparison to other studies estimating the volume of termite mounds (Nauer et al. 2018) and other biological structures, such as coral (Figueira et al. 2015, Ferrari et al. 2017, Lange and Perry 2020). Additionally, a root mean squared error (RMSE) between V_p and V_w for each round was calculated using the Metrics package in R (Hamner and Frasco 2018). A Friedman's test was then used to assess whether the precision or accuracy varied with mound size.

Field experiment

To quantify the changes in termite mound volumes over time, the effect of initial mound size and the difference in the volume of soil added to mounds between *Nitiditermes* and *Macrotermes* were considered.

Simple linear regressions were used to assess whether initial mound sizes affected the volume of soil added to a mound over time. Only termite mounds that had an overall increase in volume at the end of the 40-week experiment were considered for the simple linear regressions (*Macrotermes* = 10 mounds and *Nitiditermes* = 16 mounds). Separate linear regression models were performed for *Macrotermes* and *Nitiditermes* mounds. The data included extreme outliers so simple linear regression models with the outliers removed were also performed and included to assess whether the significance changed. Three outliers were removed from each of the *Macrotermes* and *Nitiditermes* mound datasets. The mound volume at week 0 of the experiment (V_i; L) and the volume at week 40 (V_f; L) were used to calculate the absolute rate of change in volume per week (${\Delta V_{abs}}^{-wk}$; L/week) for the 40-week time frame (t_{40}; Eq. 2).

$${\Delta V_{abs}}^{-wk} = (V_f - V_i)/t_{40} \qquad \text{Eq. 2}$$

The relative rate of change in volume (${\Delta V_{rel}}^{-wk}$; %/week) as the percentage that the mound grew each week relative to the mound's initial volume (V_i; L) was calculated (Eq.3).

$${\Delta V_{rel}}^{-wk} = \{[(V_f - V_i)/t_{40}]/V_i\} * 100 \qquad \text{Eq. 3}$$

Wilcoxon tests were used to assess the difference in the absolute and relative rate of soil volume added between *Nitiditermes* and *Macrotermes* mounds. Only termite mounds that had an overall increase in volume at the end of the 40-week experiment were considered (*Macrotermes* = 10 mounds and *Nitiditermes* = 16 mounds). The absolute (L/week) and relative (%/week) rate of change in volume were arcsine square root transformed for the Wilcoxon tests. The transformed absolute and relative rate of change in volume were the response variables and the termite genera (*Macrotermes* and *Nitiditermes*) was the predictor variable for both Wilcoxon tests.

Seasonal changes in mound volume

To quantify the seasonal changes in termite mound volume, the absolute and relative rate of change in volume for the first 8 weeks (t_8 ; wet season) and the next 32 weeks (t_{32}; majority dry season) of the experiment were considered separately by using the volume of *Macrotermes* termite mounds taken at 8 weeks (V_8; L). The volume of soil added to the termite mounds over the last 32 weeks of the experiment was calculated by subtracting the volume of the mounds at 8 weeks (V_8; L) from the volume at 40 weeks (V_f; L).

The absolute ($\Delta V_{abs8}{}^{-wk}$; L/week) and relative ($\Delta V_{rel8}{}^{-wk}$; %/week) rate of change in volume for the first 8 weeks were calculated with Eq. 4 and Eq. 5. The absolute ($\Delta V_{abs32}{}^{-wk}$; L/week) and relative ($\Delta V_{rel32}{}^{-wk}$; %/week) rate of change in volume for the next 32 weeks of the experiment were calculated with Eq. 6 and Eq. 7.

$$\Delta V_{abs8}{}^{-wk} = (V_8 - V_i)/t_8 \qquad \text{Eq. 4}$$

$$\Delta V_{rel8}{}^{-wk} = \{[(V_8 - V_i)/t_8]/V_i\} * 100 \qquad \text{Eq. 5}$$

$$\Delta V_{abs32}{}^{-wk} = (V_f - V_8)/t_{32} \qquad \text{Eq. 6}$$

$$\Delta V_{rel32}{}^{-wk} = \{[(V_f - V_8)/t_{32}]/V_i\} * 100 \qquad \text{Eq. 7}$$

Wilcoxon tests were used to assess the difference in the absolute and relative rate of soil volume added to *Macrotermes* mounds between the first 8 weeks of the experiment (wet season) and the next 32 weeks of the experiment (majority dry season). *Macrotermes* mounds were included in the Wilcoxon tests for seasonal differences because only *Macrotermes* mound volumes were measured after a period of 8 weeks. *Macrotermes* mounds that increased in volume monotonically over the experimental time were considered for the Wilcoxon tests (n = 7). The rates of change in absolute (L/week) and relative (%/week) volumes were then arcsine square root transformed for the Wilcoxon tests. The transformed absolute and relative rate of change in volume were the response variables and the number of weeks (0 – 8 and 8 – 40 weeks) was the predictor variable for both Wilcoxon tests. All analyses were performed in R Statistical Software (v4.2.0, R Core Team 2022).

RESULTS

Accuracy and precision assessment

The photogrammetric technique consistently over-estimated the mound volumes determined through water displacement (Fig. 2). However, the volume was precise with low SDs and CVs of the estimated volume for the small (SD: 0.02 L; CV = 0.61%; Fig. 2a), medium (SD: 0.03 L; CV = 0.46%; Fig. 2b), and large (SD: 0.10 L; CV = 0.21%; Fig. 2c) artificial mounds. The RMSE between V_p and V_w for each round was low at 0.86. In all three sizes of artificial mounds, there was less than a 5% difference in volume between V_w and V_p across all five rounds (Fig. 3). A Friedman's test showed that there was no significant difference in volume difference (%) with mound size $X^2_F(4) = 3.47$, $p > 0.05$.

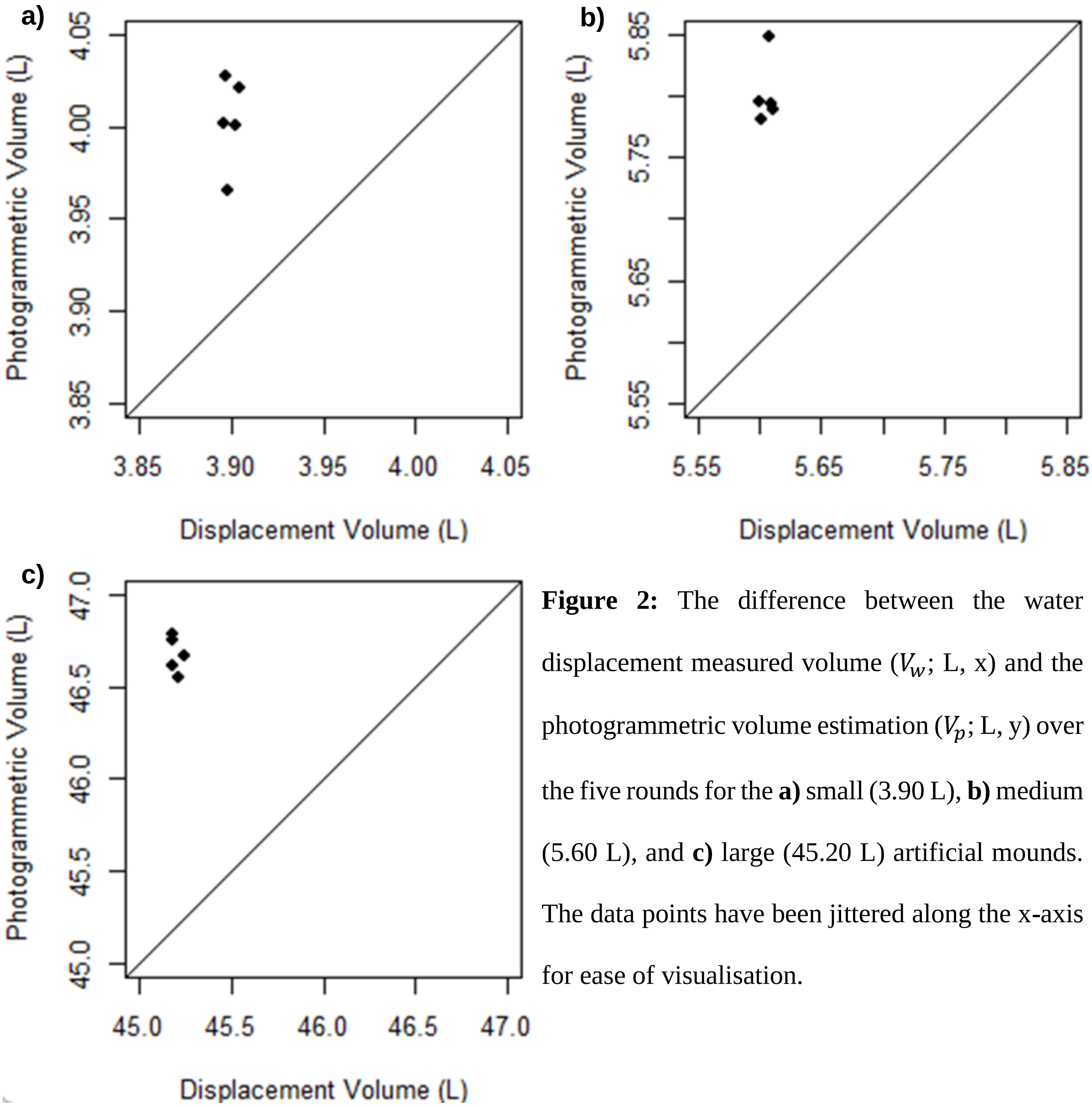

Figure 2: The difference between the water displacement measured volume (V_w; L, x) and the photogrammetric volume estimation (V_p; L, y) over the five rounds for the **a)** small (3.90 L), **b)** medium (5.60 L), and **c)** large (45.20 L) artificial mounds. The data points have been jittered along the x-axis for ease of visualisation.

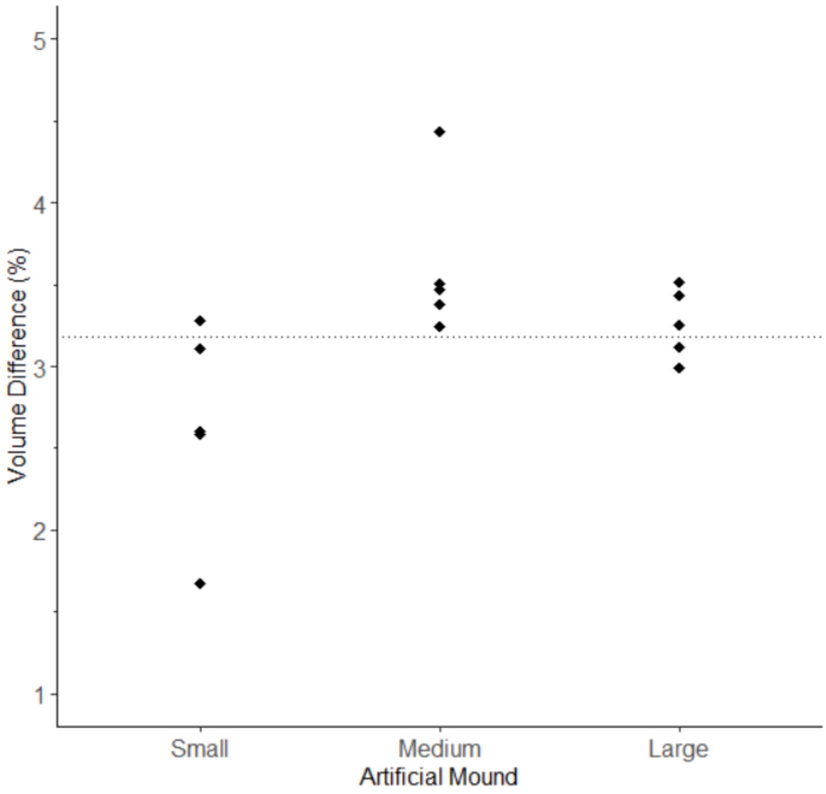

Figure 3: The percentage of the volume difference ($V_{d\%}$; %) between the water displacement measured volume (V_w; L) and the photogrammetric volume estimation (V_p; L) over the five rounds for the small (3.90 L), medium (5.60 L), and large (45.20 L) artificial mounds. The dashed line represents the average (3.18%) of all volume differences regardless of artificial mound size.

Change in termite mound volume over time

Over the 40-week experimental period, 10 out of 13 (~ 80%) *Macrotermes* mounds and 16 out of 20 (~ 80%) *Nitiditermes* mounds increased in volume, while ~ 20% of the mounds decreased in volume for both genera (Fig. 4).

There was no significant relationship between initial mound volume (V_i; L) and the absolute rate of change in the volume ($\Delta V_{abs}{}^{-wk}$; L/week) over 40 weeks for *Macrotermes* (Fig. 5a) in both the linear regression with the full dataset ($r^2 = 0.09$, $b = - 0.01$, $F_{1,8} = 0.80$, $p > 0.05$) and with outliers removed ($r^2 = 1.75 \times 10^{-5}$, $b = -2.81 \times 10^{-5}$, $F_{1,5} = 8.75 \times 10^{-5}$, $p > 0.05$). There was a significant positive relationship between initial mound volume (V_i; L) and the absolute rate of change in volume (${\Delta V_{abs}}^{-wk}$; L/week) over 40 weeks for *Nitiditermes* (Fig. 5b) in both the linear regression with the full dataset ($r^2 = 0.27$, $b = 0.01$, $F_{1,14} = 5.18$, $p = 0.04$) and with outliers removed ($r^2 = 0.43$, $b = 0.003$, $F_{1,11} = 8.19$, $p = 0.02$).

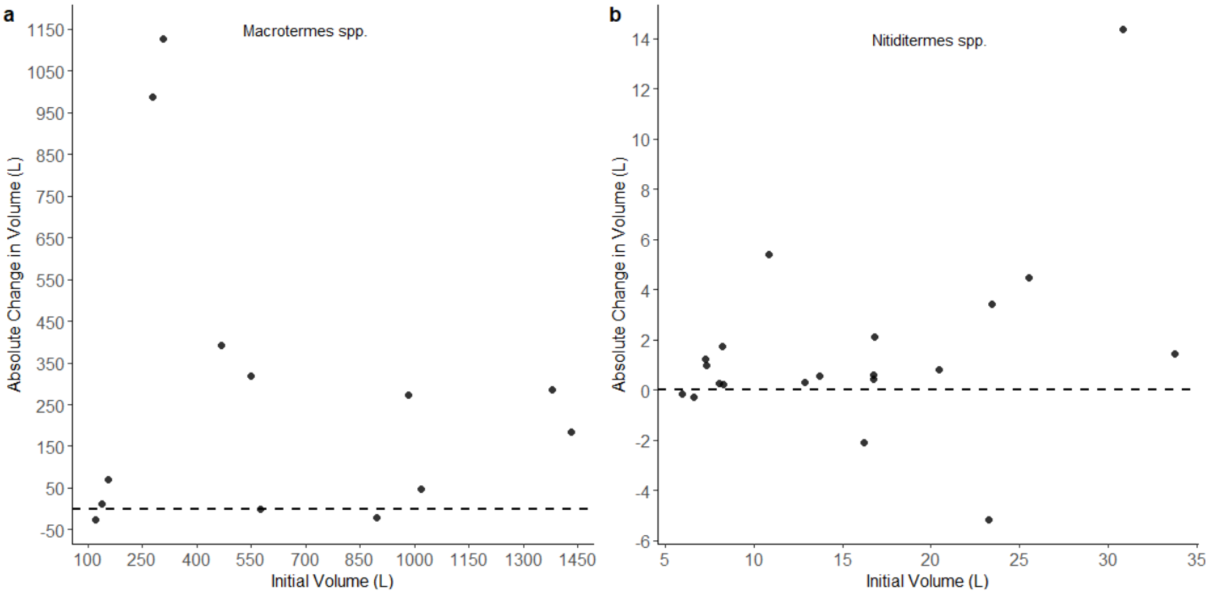

Figure 4: The absolute change in volume ($V_f - V_i$; L) that occurred over 40 weeks in **a)** *Macrotermes* (n = 13) and **b)** *Nitiditermes* (n = 20) termite mounds according to their initial volume (V_i; L). The dashed line indicates no change in volume.

A Wilcoxon test showed that the difference between the absolute rate of change in volume (${\Delta V_{abs}}^{-wk}$; L/week) added to *Macrotermes* and *Nitiditermes* mounds over 40 weeks was statistically significant W = 159, $p < 0.01$ (Fig. 6a). A Wilcoxon test showed that the difference between the relative rate of change in volume (${\Delta V_{rel}}^{-wk}$; %/week) added to *Macrotermes* and *Nitiditermes* mounds over 40 weeks was statistically significant W = 130, $p < 0.01$ (Fig. 6b).

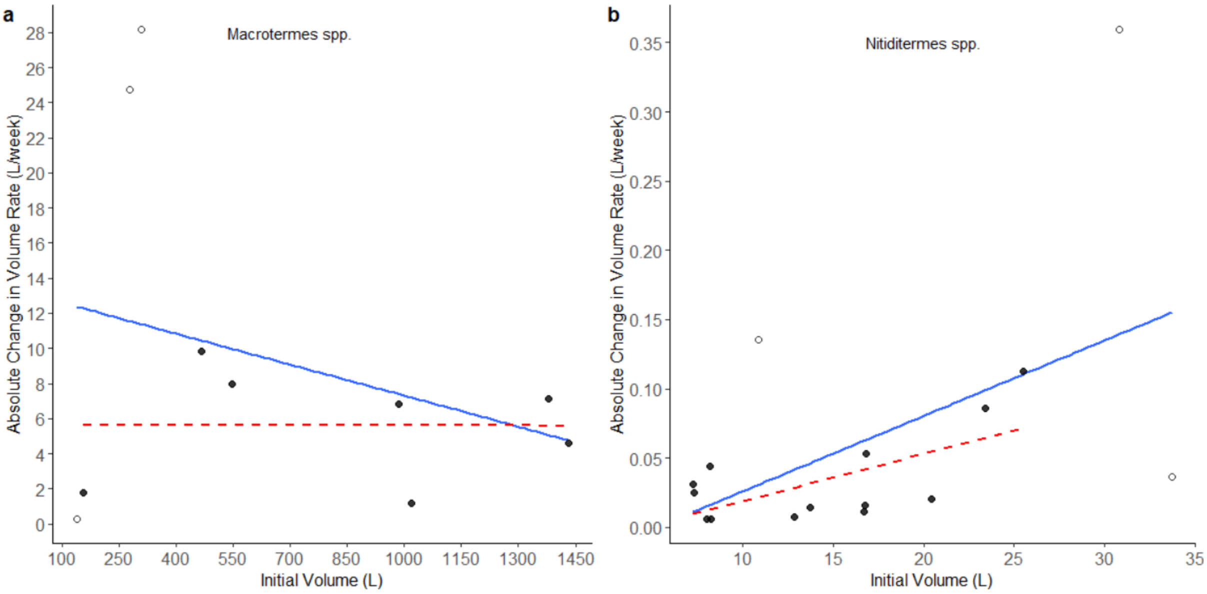

Figure 5: The relationship between initial mound volume (V_i; L) and the absolute rate of change in volume ($\Delta V_{abs}{}^{-wk}$; L/week) after 40 weeks distinguished for **a)** *Macrotermes* (n = 10) and **b)** *Nitiditermes* (n = 16). The solid line represents the linear regression model with the full dataset while the dashed line represents the linear regression model with outliers removed. The removed outliers are represented by hollow points.

Pore space assessment

The pore space for *Nitiditermes* mounds was variable but less than a litre of pore space was present on average (Table 1). The average pore space was 0.16 L for every 1 L (16.40%) of soil in a mound. The average density of soil was 1207.50 g. L^{-1}. *Macrotermes* had a larger range in mound volume and soil weight added to a mound per week than *Nitiditermes* over the experimental time (Table 2).

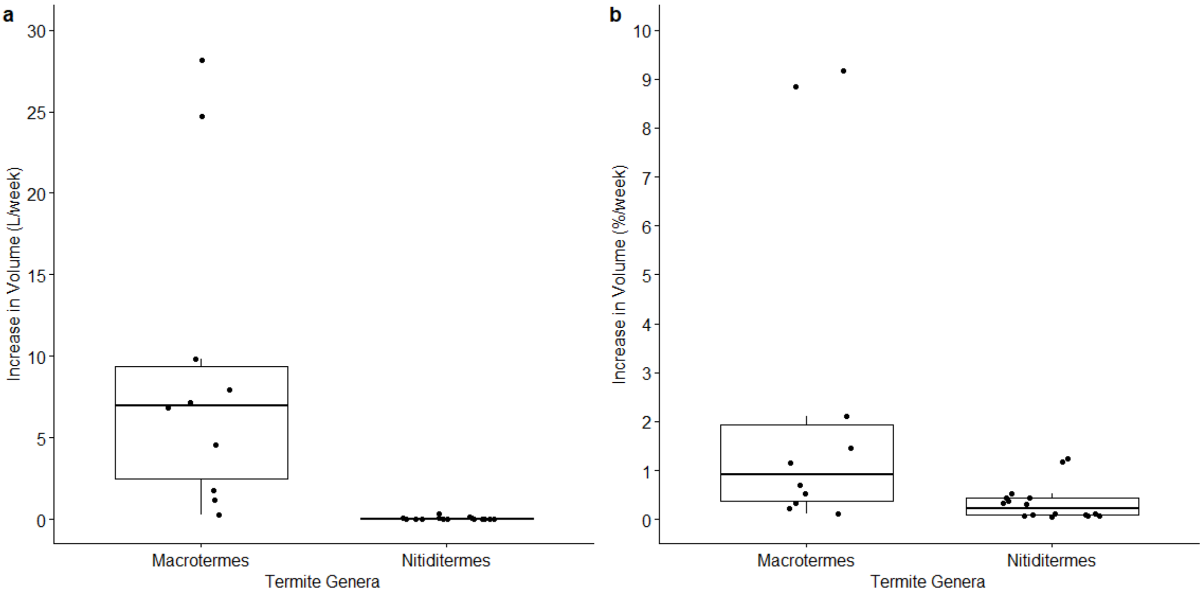

Figure 6: The **a)** absolute ($\Delta V_{abs}{}^{-wk}$; L/week) and **b)** relative ($\Delta V_{rel}{}^{-wk}$; %/week) rate of change in volume per week of *Macrotermes* (n = 10) and *Nitiditermes* (n = 16) mounds that had an overall increase in volume over the 40 weeks of the experiment.

Table 1: The two *Nitiditermes* mounds that were cut in half (cross-sectionally) and their photogrammetric estimated volumes at 40 weeks (L), the volume after they were crushed (L), the pore space (L), and the density (g. L^{-1}) for each mound. Density was calculated using the dry weight of a soil sample from each mound.

Mound	**Estimated Volume (L)**	**Crushed Volume (L)**	**Pore Space (L)**	**Density (g. L^{-1})**
1	4.48	4.20	0.28	1188
2	1.97	1.20	0.77	1227

Table 2: The minimum and maximum amount of soil added to *Macrotermes* and *Nitiditermes* mounds per week (kg. week^{-1}) and the associated volume range (volume at week 0 to week 40) of each mound (L).

Genus	**Weight (kg. week^{-1})**		**Mound Volume Range (L)**
Macrotermes	**Minimum:**	0.36	139.19 – 151
	Maximum:	34	307.40 – 1434.01
Nitiditermes	**Minimum:**	< 0.001	8.31 – 8.55
	Maximum:	0.44	30.79 – 45.16

Seasonal changes in volume

A Wilcoxon test showed that the difference between the absolute (W = 18.50, p > 0.05; Fig. 7a) and relative (W = 19.50, p > 0.05; Fig. 7b) rate of change in volume added to *Macrotermes* mounds over the first 8 weeks ($\Delta V_{abs8}{}^{-wk}$; $\Delta V_{rel8}{}^{-wk}$; November 2019 – January 2020) were not statistically significantly different from the next 32 weeks ($\Delta V_{abs32}{}^{-wk}$; $\Delta V_{rel32}{}^{-wk}$; January 2020 – September 2020) of the experiment.

The average bait consumption score (Fig. 8a), as a proxy for termite activity, was ~ 1.4 for the first 8 weeks and ~ 1.2 for the next 32 weeks of the experiment. The bait score for June 2020 was disregarded because it was left out in the field from March 2020 due to COVID-19 regulations. A range of 0 – 103.60 mm. week^{-1} of rainfall fell during the first 8 weeks and 0 – 66.6 mm. week $^{-1}$ in the following 32 weeks of the experiment (Fig. 8b).

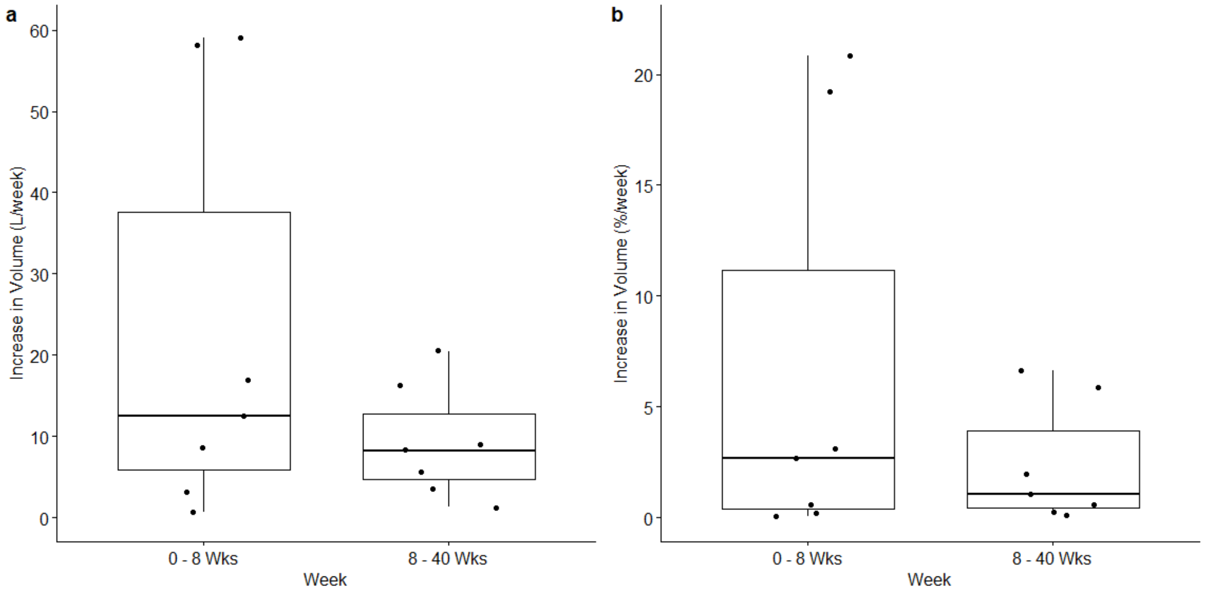

Figure 7: The difference between the **a)** absolute (L/week) and **b)** relative (%/week) rate of change in volume over the first 8 weeks ($\Delta V_{abs8}{}^{-wk}$; $\Delta V_{rel8}{}^{-wk}$; 0 – 8 Wks) and the next 32 weeks ($\Delta V_{abs32}{}^{-wk}$; $\Delta V_{rel32}{}^{-wk}$; 8 – 40 Wks) of the 40-week experiment for *Macrotermes* mounds (n = 7) that increased in volume monotonically.

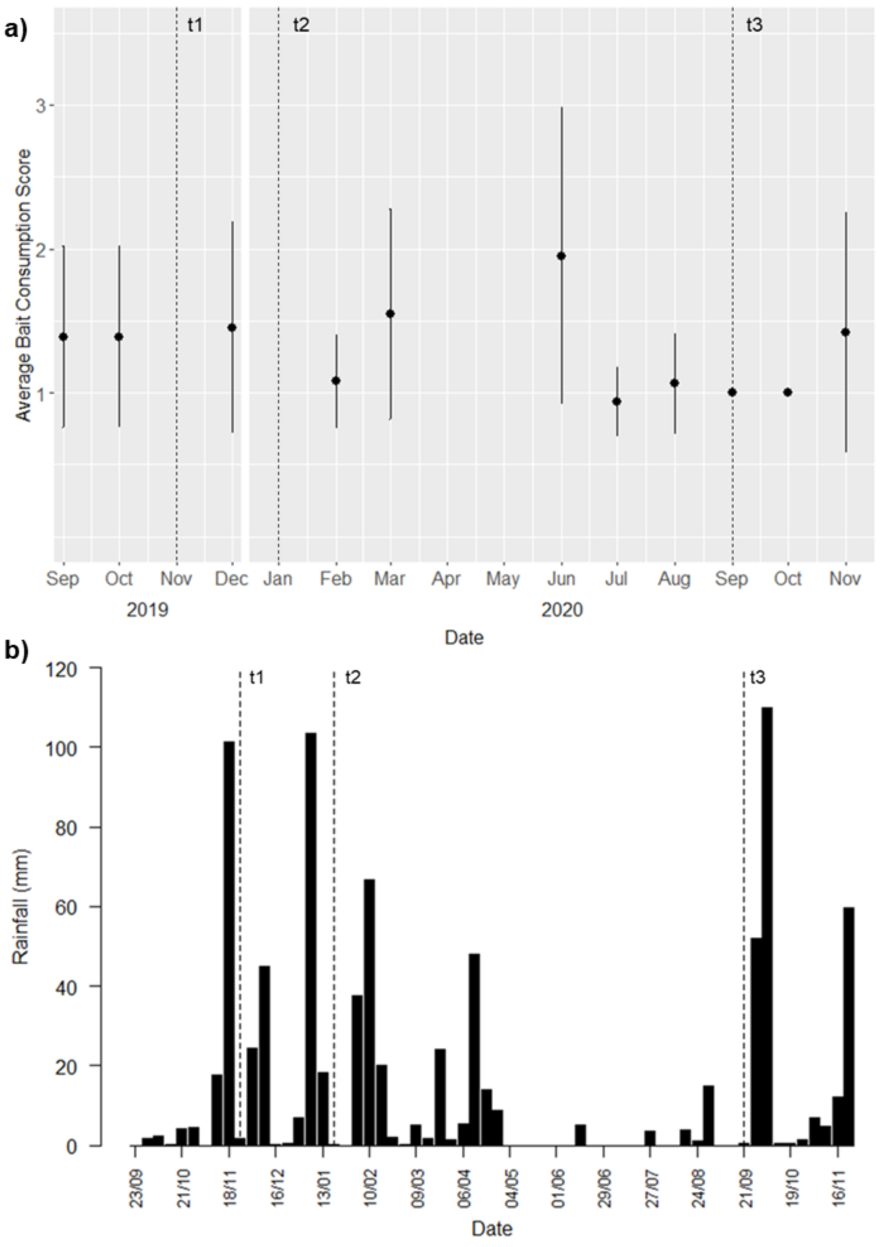

Figure 8: a) The mean bait consumption score per month and standard deviations (SDs) on the control plots. Bait consumption is a proxy of termite activity and was scored depending on how much of the bait was consumed 0 – 25% (score 1); 25 – 49% (score 2); 50 – 75% (score 3); 75 – 94% (score 4); 95 – 100% (score 5). Termite activity data for November 2019, and January, April, May 2020 were not available. **b)** The amount of rainfall (mm) per week over a 62-week period (23 September 2019 – 23 November 2020) at Wits Rural Facility (WRF) before and after the experiment was conducted. In both figures the dashed lines represent when mound volume was recorded at the start of the experiment (t1 = Week 0), after 8 weeks (t2 = Week 8), and at the end of the experiment (t3 = Week 40).

DISCUSSION

This study demonstrates that 3D photogrammetry can be used as a non-invasive technique to estimate the change in volume of termite mounds for multiple termite genera. We estimated the volume of termite mounds at different time frames and were able to quantify the addition of volume to a termite mound as compared to its initial size. We showed that there were differences in the addition of volume and weight of soil moved between two termite genera. Lastly, we were able to assess that there was a seasonal difference in the rate of change in the volume of termite mound growth.

Photogrammetric technique

The accuracy and precision of the photogrammetric technique to estimate the volume of termite mounds was evaluated by using artificial metal mounds of differing size and measuring their volume using water displacement. Despite the photogrammetric technique consistently overestimating the volume of the artificial mounds, the volume estimated was very precise with SDs (0.02 – 0.10 L or 20 – 100 ml), CVs (0.21 – 0.61%), and a small RMSE (0.86); less than a 5% difference between the estimated mound volume and the volume calculated by water displacement. To our knowledge, only one other study has used photogrammetry to estimate the volume of termite mounds (Nauer et al. 2018). In an Australian coastal savanna woodland, Nauer et al. (2018) highlighted photogrammetry as a superior technique to estimate mound volume over more traditional methods, such as cylinder, spheroid, and cone geometrics. They observed that compared to photogrammetry, cylinder geometrics overestimate (by 190 ± 1.50%; RMSE = 70.50), while cone (by 3.60 ± 5%; RMSE = 23.50), and hemi-spheroid (by 7 ± 4.50%; RMSE = 21.30) geometrics underestimate the volume of termite mounds. Nauer et al. (2018) used rocks to validate their photogrammetric technique and found that it underestimated rock volumes with the largest error at 3.50% and a CV of 0.60% when compared to a reference water displacement volume of the rocks. Our photogrammetric technique overestimated artificial metal mound volumes, but our average overestimation of 3.18%, CVs (0.21 – 0.61%), and small RMSE of 0.86 was comparable to Nauer et al. (2018)'s underestimation value, CV, and small RMSE of 0.17. Additionally, we found that overestimation did not vary with artificial mound size. Furthermore, studies using SfM photogrammetry to estimate the volume of corals and sponges also had small CVs of less than 7.80% (Lavy et al. 2015) and 2% (Ferrari et al. 2017).

Water displacement has long been regarded as a highly accurate measurement for volume (Scherle 1970) and has been used in estimating volume for benthic organisms (Jokiel and Maragos 1978). Detachment of the coral or sponge for water displacement volume measurements, as previously done by Rützler (1972), should be the last resort and previous studies demonstrated that photogrammetry 3D modelling is equivalent to the accuracy of measuring the volume of corals by water displacement (Cocito et al. 2003, Lavy et al. 2015). Lavy et al. (2015) used videos of coral to create a 3D model and estimate their volume with only a deviation of between 1.40 – 12.80% when compared to water displacement. Cocito et al. (2003) found that there was a deviation of 0.20 – 4.10% dependent on different coral morphologies and when using only 8 – 12 still frame images. Our study has comparable error values and variation to these previous studies.

We also made use of consistent scale reference points (i.e., the rulers that have predefined measurements and are present in all the photogrammetry projects), similar to Raoult et al. (2017) who defined a potential error of ~ 15% when assessing the growth of corals. Bennecke et al. (2016) conducted a similar study to Raoult et al. (2017) on corals, but they made use of inconsistent scale reference points (i.e., markings that had no predefined measurements and were not present in some of the projects) which caused the error to increase to up to 28% over an 8-year time frame. Given that our study had consistent scale reference points and the change in mound volumes that were measured between time frames were larger (> 10% for *Nitiditermes* and *Macrotermes* mounds with the lowest change in volume) than the overestimation errors (< 5%), we are confident that our photogrammetric technique was precise in estimating the volume of termite mounds in litres with a slight deviation of between 20 - 100 ml.

The overestimation could be due to errors associated with the manual placement of the reference scale points and cropping the meshes or DSMs to ensure the termite mound was only included in the volume estimation and not the surrounding ground plane. Raoult et al. (2017) found that ~ 2% of the error could be attributed to these same user-based sources of error with the rest of the error remaining unexplained. However, these two sources of error are ingrained in photogrammetric techniques, including the one used in our study, and are unlikely to be addressed soon (Raoult et al. 2017). The error associated with the usage of different observers, (e.g., Raoult et al. 2017), was unnecessary in our study as the same observer conducted the photogrammetry procedure. Herein, the focus is on a biological aspect of mound building, so the change in volume over time is more important than the absolute change in mound volume. As our technique consistently overestimates volume with high precision it can be regarded as accurate in any study that seeks to investigate the temporal change in mound volumes. On King George Island and sub-Antarctic Marion Island, de Bruyn et al. (2009) found that their 3D photogrammetric technique consistently overestimated the measured mass of seals (6.59 ± 1.52%) but the consistent overestimation allowed for a correction factor to be applied to successfully estimate the mass of seals. Therefore, with such consistent overestimation, correction factors can be easily applied, and our photogrammetric technique has the potential for broader applications that require precision as well as high accuracy.

Changes in termite mound volume

Change in termite mound volume was successfully quantified with this photogrammetric technique. Repeated measurements allowed us to observe that termite mounds changed in volume either monotonically or non-monotonically over time. Most (~ 80%) of the termite mounds increased, while some (~ 20%) decreased in volume over the 40-week study period. The dynamic between mound construction by termites that adds volume and mound erosion (principally rainfall) that removes volume was most likely the cause of volume changes. When considering the dynamic between construction and erosion of termite mounds it is important to analyse data from different termite genera separately because of the difference in mound building biology and colony size (Gosling et al. 2012).

We found that initial mound size only had a significant effect on the volume of soil added to *Nitiditermes* but not to *Macrotermes* mounds. Only *Nitiditermes* mounds with a larger initial volume had a larger increase in the absolute rate of change in volume (L/week). Therefore, larger mounds added more volume per week than smaller mounds. Colony size has been associated with the size and height of termite mounds (Josens and Soki 2010, Ocko et al. 2019). It has been shown that larger termite colonies build larger mounds across multiple taxa, including *Macrotermes* and *Nitiditermes* (Josens and Soki 2010, Wango and Josens 2011, Ocko et al. 2019). Therefore, *Macrotermes* mounds with a larger initial volume were expected to add more volume per week than smaller mounds. It is likely that the small sample size for *Macrotermes* (n = 10) mounds in our study explains the non-significant relationship. *Macrotermes* increased in volume at a higher rate and added larger amounts of soil to their mounds than *Nitiditermes*. *Nitiditermes* can add an average of 72.50 g and *Macrotermes* an average of 11.17 kg of soil per week to their mounds. The mean weight of soil added to mounds over the 40-week study for *Nitiditermes* was 2.91 kg (average V_f = 18.72 L) and *Macrotermes* was 446.61 kg (average V_f = 1040.73 L).

We acknowledge the presence of pore space inside termite mounds in the form of tunnels and galleries and thus our weight calculations are overestimations. In our assessment of pore space, to account for these overestimations, we found that the average pore space was 0.164 L for every 1 L of soil in a mound. Therefore, 16.40% of a mounds volume was attributed to pore space. The average for the last volume (taken at 40 weeks) for *Nitiditermes* mounds was 18.72 L and *Macrotermes* mounds was 1040.73 L. However, the morphology (i.e., the external structure and the internal architecture) of termite mounds is dependent on the genus of termite being considered (Korb 2010, Nauer et al. 2018). We expected to see a significant relationship between the volume added to a mound and the initial mound volume for *Macrotermes* as colony size has been associated with mound height and size (Josens and Soki 2010, Ocko et al. 2019). A small sample size or non-linear relationship with initial mound volume could also explain the non-significant relationship as *Macrotermes* mounds could have been in different stages of growth. The external structure of a mound is said to be dependent on the reproductive age and size of the colony and the internal architecture is to help regulate humidity, temperature, gas flow and the microclimates specialised for brooding and cultivating fungi (Darlington and Dransfield 1987, Josens and Soki 2010, Muvengwi et al. 2018c, Ocko et al. 2019, Fagundes et al. 2021). As the morphology of termite mounds differs among genera it would be more beneficial to assess the porous space of mounds per genus and apply the appropriate calculations. Nauer et al. (2018) used photogrammetry and X-ray CT scanning to determine the porosity and bulk density of termite mounds, which is more reliable than crushing mounds. For logistical reasons this was not possible in this study and *Macrotermes* mounds were too large to easily cross-section for our mound crushing method. Therefore, we acknowledge the pore space calculated in our study is likely an underestimate for *Macrotermes*.

Several studies have highlighted the importance of termites as soil engineers and the organo-chemical (Jouquet et al. 2005, 2011, Kristensen et al. 2019) and physical effects (Jouquet et al. 2011, Cheik et al. 2019) that they have on soil through activities such as constructing mounds and foraging. As our study highlights, the soil moved to construct their mounds is immense and mounds have been estimated to weigh between 100 - 2.4×10^6 kg. ha^{-1} (Meyer 1960, Lee and Wood 1971). Coupled with the large density of mounds in a landscape, where they can influence up to 30% of the area, termite mounds have a significant impact on the soil status of an ecosystem (Wood 1988, Davies et al. 2014b, Muvengwi et al. 2018a). In Kruger National Park, South Africa, Davies et al. (2014b) found termite mound densities, in an area near our own study site, can occur between 1.52 to 1.87 mounds ha^{-1} on landscape crests in a savanna.

Erosion of termite mounds also contributes to the redistribution of soil nutrients in the ecosystem (Muvengwi et al. 2016, 2018b, Shanbhag et al. 2017). The erosion of termite mounds caused by rainfall is dependent on rainfall type and intensity (Bonell et al. 1986, Shanbhag et al. 2017). For example, the loss of soil through erosion from termite *M. bellicusus* has been reported at ~ 3-ton ha^{-1}. y^{-1} in the Sudanian zone, Ivory Coast, which received an average rainfall of 1100 mm. $year^{-1}$ during their study (Lepage 1984). Termites are most active in the wet season, linked to building activity during or soon thereafter (Turner et al. 2006). We found that there was no difference in the rate of change in *Macrotermes* mound volume between the first 8 weeks of the experiment and the next 32 weeks.

However, some weeks (weeks 9 – 20 of the experiment) included in the 32-week time frame fall in the wet season (typically occurring between November and April at WRF). The average bait consumption score, as a proxy for termite activity, and weekly rainfall (mm) were higher during these weeks (weeks 9 – 20) compared to weeks (weeks 21 – 40) falling in the dry season (typically occurring between May and October at WRF). As termite activity is higher in the wet season (Turner et al. 2006), the inclusion of wet season weeks in calculations for the dry season likely reduced contrast in termite building activity (i.e., rate of change in mound volume) between the two seasons.

Quantifying the volume change of termite mounds and previous studies on erosion (Lepage 1984, Aloni and Soyer 1987) demonstrates the volumes of nutrient rich soil being cycled through the ecosystem by termites and highlights their importance as influential soil engineers that promote spatial heterogeneity in ecosystems (Moe et al. 2009, Sileshi et al. 2010, Muvengwi et al. 2017). In KwaZulu-Natal, South Africa, Gosling et al. (2012) found that the erosion from termite mound soil can influence the lawn grasses found in a savanna. In Kruger National Park, South Africa, Davies et al. (2016b, 2016a) found that termite mounds can exert a strong influence on herbivory through the creation of spatial heterogeneity by promoting vegetation patchiness.

Limitations and future studies

Our study demonstrates that photogrammetry is a suitable technique for estimating the volume of termite mounds because of its non-invasive, quick application in the field. The quantification of bioturbation components of termite mound volume (i.e., growth or building) were successfully assessed in this study. However, it should be noted that our sample size of artificial mounds was low, and the largest volume of an artificial mound (42.50 L) was not representative of the largest termite mound volume (1434.01 L) in the field. Logistical constraints such the ability to build large enough artificial mounds and the ability to perform water displacement measurements on said mounds limited their inclusion. For future research, larger artificial mounds, and other forms of measurement such as laser or CT scanning (Nauer et al. 2018) could be used to assess the precision and accuracy of the photogrammetric technique in use. If feasible, laser scanning can be used as a ground-truth measurement that can be compared with estimations from photogrammetric techniques (McKinnon et al. 2011, James and Robson 2012). However, CT scanning requires expensive equipment and its use on termite mounds is limited by the scanner capacity (Nauer et al. 2018). The photogrammetric technique used in our study also had an upper limit for *Macrotermes* mounds and very large, vegetated mounds could not be considered when estimating mound volumes. The consideration of these large mounds is important because of the substantial amount of soil that could be translocated by the colonies that inhabit them. In future studies, using an unmanned-arial-vehicle (UAV) in conjunction with light detection and ranging (LiDAR) could assist in obtaining the volume of these large mounds. Furthermore, UAVs could be used to obtain images in conjunction with a more traditional SfM photogrammetric technique, as presented in our study, to allow for the inclusion of these large mounds as well as the smaller mounds in a landscape that could have been missed when using LiDAR. Davies et al. (2014b) used LiDAR to obtain data on the densities of mounds in the Kruger National Park, but mounds of less than 2 m in height could not be detected. Remondino et al. (2011) outlines the use of UAVs in large-scale applications such as forestry and agriculture to map landscape surfaces but also suggests that UAVs can be used for small-scale applications in conjunction with the appropriate photogrammetric technique.

For the seasonal difference in the rate of change in mound volume, there were no data for after 16 weeks, which was originally intended, due to COVID-19 regulations preventing fieldwork. The 16-week mark would have subdivided the wet and dry seasons more appropriately. For future studies, a similar workflow or even smaller time frames (e.g., weekly basis) can allow for additional volume metrics to be considered, such as the change in mound shape over time, the response of building activity related to high rainfall events, and non-linear mound growth.

CONCLUSION

Our study is the first to apply a 3D photogrammetric technique to demonstrate detailed insights into the temporal changes in termite mound volumes to quantify bioturbation. We further demonstrate the suitability of photogrammetry for termite mound volume estimation. Using a rigorously tested photogrammetric technique, we were able to show that on average *Nitiditermes* can move 2.91 kg (average V_f = 18.72 L) and a *Macrotermes* colony can move 446.61 kg (average V_f = 1040.73 L) of soil over 40 weeks to build their mounds. These estimates are conservative as very large mounds are likely show more substantial amounts of soil movement as colony sizes increase with mound size. We highlight the importance of termites as soil engineers, in bioturbation in a savanna ecosystem. In a future with climate change and the impacts of land use change, it is becoming increasingly important to quantify the influence that functionally important soil engineers have on ecosystems to determine their potential impact on conservation and restoration schemes.

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CHAPTER 2: SUPPLEMENTARY MATERIAL

Field experiment

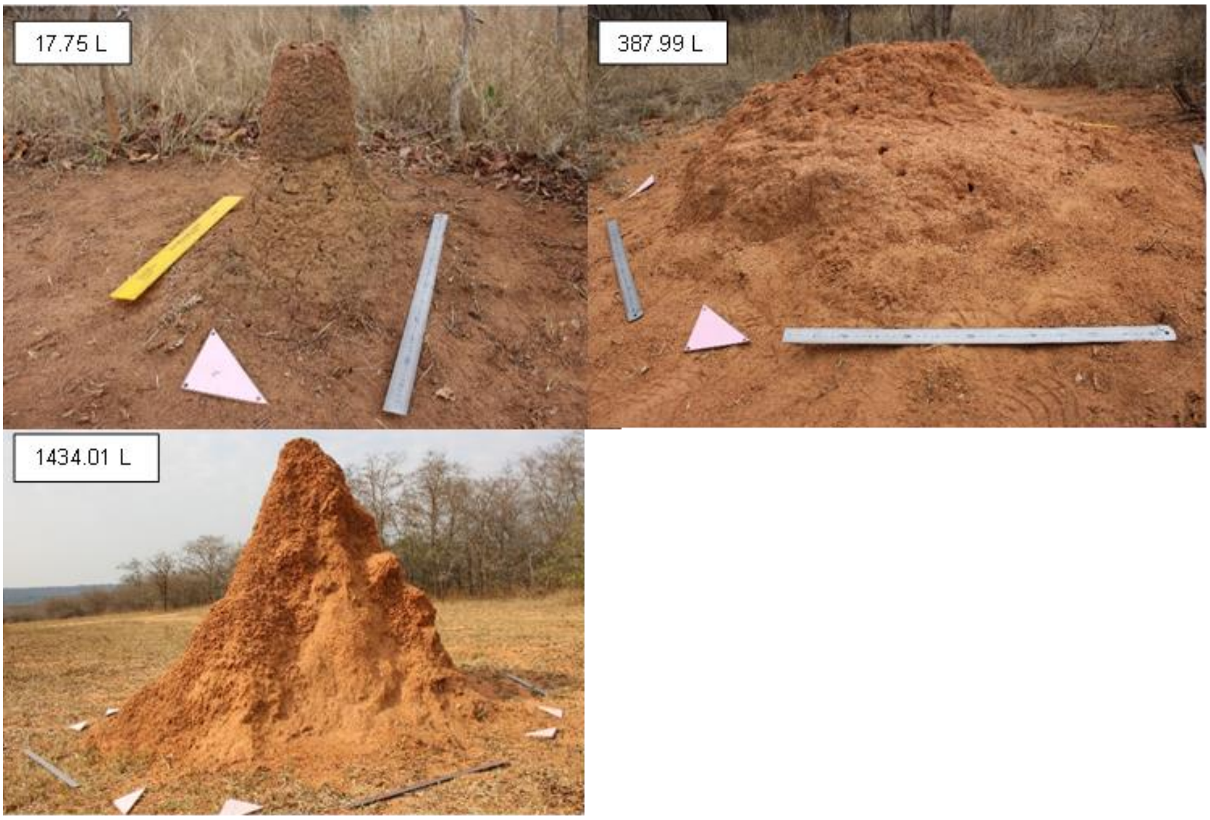

Figure S1: Images depicting three mounds that were used in the study and how the triangular markers and metal rulers were placed around the mounds for the photogrammetry. The markers were used to aid in feature detection and photographic orientation. The rulers were used as a scale for the volume estimation. The images were taken in September 2020 (Week 40).

Photogrammetric volume estimation

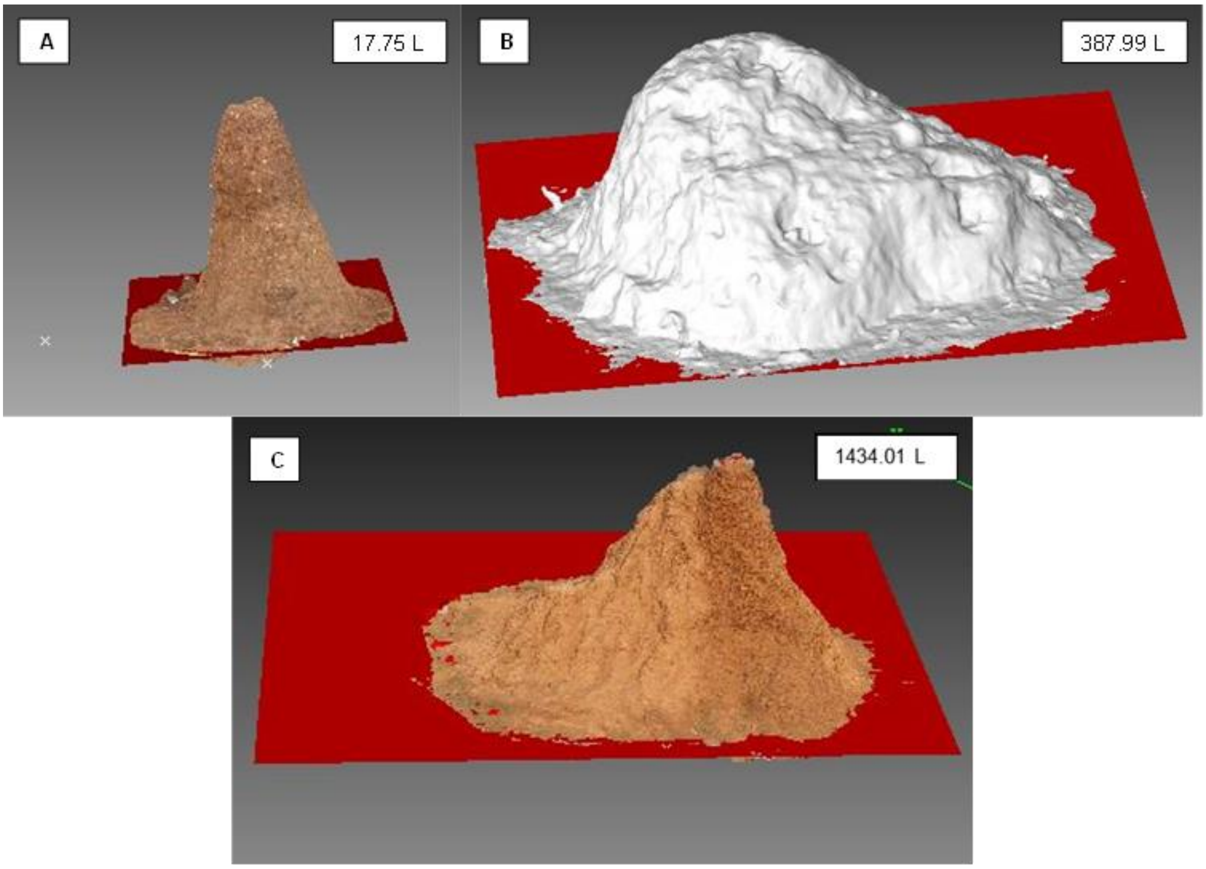

Figure S2: Images depicting the three-dimensional models of the three mounds (pictured in Fig. S1). **A)** illustrates the two points (white crosses) that were assigned the appropriate distance between two points manually selected on a ruler from at least 10 digital photographs of the project that was used to designate real-life scale accuracy for the volume estimation in the modelling process. **B)** illustrates the dense surface modelling with triangulation that reconstructed the three-dimensional surface of the mound. **C)** illustrates the surface textures (also seen in **A**) that were generated for each mound via the photographs to help ascertain which part of the dense surface model was the mound versus the ground surrounding the mound to enable the cropping of the immediate ground surface to be accurate. Illustrated in all three panels is the plane (in red) that was generated in the three-dimensional space as the best fit for SmartPoints ® that represented the ground immediately around the mound.

Pore space assessment pilot study

Termite mounds were crushed to take the galleries and tunnels (pore space) inside the mounds into account. As there are two genera of termite present (*Nitiditermes* and *Macrotermes*) in this study, at least one mound per genus needed to be crushed. Three *Nitiditermes* mounds, and one *Macrotermes* mound were chosen to be crushed giving an overall sample size of four. The mounds were removed from the ground and transferred into rigid containers to be crushed to the same soil consistency. A volumetric beaker was then used to manually measure the volume of each mound. The mounds were crushed after the last set of photographs were taken of them at 40 weeks so that the last (40 week) volume estimated by PhotoModeler ® Premium software could be compared to the crushed mound volume to calculate the pore space inside each mound. The mound volume calculated from the crushed soil was larger than the volume estimated by photogrammetry. Which is a consequence of the limitation to this crushing process; that when the mounds were being removed from the ground it was difficult to control the inclusion of soil that was below ground level.

Pilot study for accuracy and precision assessment

Building sand was used to create artificial mounds to assess whether the chosen photogrammetric procedure was accurate and precise. To achieve this, building sand was placed into a translucent rectangular rigid container and the volume of soil was calculated (length x width x height) by manual measurements. After the volume was calculated, the sand was slowly poured out to create a mound-like structure (Fig. S3). Three different mound sizes (small = 3.21 L, medium = 15.87 L, and large = 36.14 L) were used to account for the different mound sizes that would be observed in the field experiment. For logistical reasons, a mound size of only up to 40 L could be used in this pilot study. The mounds were constructed on a flat surface so that the ground plane was level. Using the chosen photogrammetric technique outlined in the materials and methods of this paper, the volume was estimated using

PhotoModeler ® Premium software. The measured volume and the volume estimated by photogrammetry were then compared to assess if there were any differences in volume. The photogrammetric procedure continually overestimated the volume of the mounds. This overestimation increased with mound size. It is suspected that when the sand was placed in the rigid container to calculate its volume, it was likely compacted more than when the sand was poured out into a mound shape. Therefore, an experiment where the mounds (i.e., metal mounds) could not be compacted under their own weight was conducted.

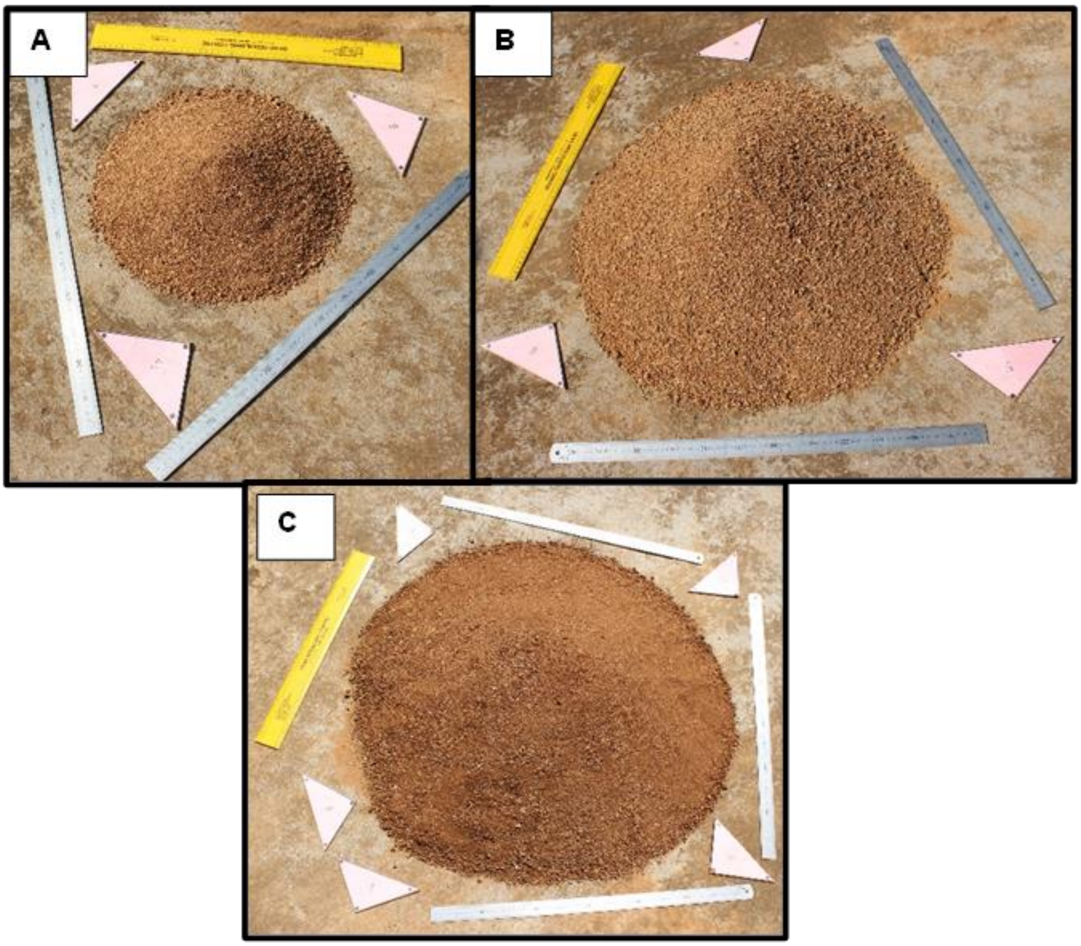

Figure S3: The three building sand mounds **A)** small (3.21 L), **B)** medium (15.87 L) and **C)** large (36.14 L) used in the pilot study. Five projects were conducted for each mound to assess the precision of the photogrammetric process. For scale, each metal ruler was 60 cm in length.

CHAPTER 3:

THE POTENTIAL FOR ANTS TO INFLUENCE TERMITE-MEDIATED BIOTURBATION IN AN AFRICAN SAVANNA

ABSTRACT

Termites and ants are considered prominent ecosystem engineers that perform ecosystem processes which impact the ecological function of an ecosystem. Termites are important bioturbators and mix soil through their foraging activities and construction of epigeal structures. Bioturbation of soil is an important process because it regulates essential ecological functions such as the infiltration and diffusion of water, the quality of water run-off, soil erosion resistance, and nutrient cycling. Ants are important predators of termites and may have a top-down effect on termite-mediated bioturbation. However, few studies have assessed the influence that ants have on limiting the populations and activity of termites at large enough scales. The aim of this study was to determine the effect of ants and termites on the movement of soils in the upper 30 cm layer of the soil profile in a savanna. We used large-scale suppression experiments to reduce the abundance of ants and termites. We then measured the movement of an optical tracer from the surface vertically down and laterally. We predicted that bioturbation would be reduced when the abundance of termites was suppressed and promoted when ant abundance was suppressed as termites are released from predation. Our study showed that at certain depths and time frames, termite-mediated bioturbation was influenced by changes in either ant or termite abundance. The suppression of ant abundance caused a larger amount of soil movement, and the suppression of termite abundance lowered the amount of soil movement. Therefore, ants can have a potential top-down control on termite-mediated bioturbation via predation pressure. Understanding the interaction between ants and termites, prominent ecosystem engineers, is crucial in a future of climate change, habitat fragmentation and a likely decline in predator diversity. The influence of ants on termites-mediated bioturbation and nutrient cycling is considerable in this nutrient-poor ecosystem.

Key words:

ants, bioturbation, ecological function, ecosystem engineers, optical tracer, predation, soil movement, termites, top-down control

INTRODUCTION

Savannas are diverse ecosystems that are found throughout the tropics and consist of a C4 dominated grassland with scattered trees and or shrubs (Solbrig 1996, Ratnam et al. 2011). The African continent has an extensive area of savanna that covers about 50% of the African territory (Menaut 1983, Campbell 1996). Termites perform a key role in the dynamics of an African savanna because their biomass is comparable to that of ungulates and African megaherbivores (Moe et al. 2009). Therefore, establishing what affects the activity of termites in the savanna ecosystem can provide important insight for conservation efforts. Termites are ecosystem engineers because of the integral role they play in affecting the abiotic and biotic components of the surrounding ecosystem (Jones et al. 1994, 1997, Jouquet et al. 2006). The abiotic environment can be affected by the creation of spatial heterogeneity when resources like soil nutrient availability are changed (Jouquet et al. 2006). The alteration or creation of habitats for other organisms in the ecosystem, by indirectly or directly mediating the availability of resources through spatial heterogeneity, causes biotic changes in the environment (Jones et al. 1994, 1997, Jouquet et al. 2006). For example, the creation of nutrient hotspots on termite mounds can cause changes in the vegetation pattern by promoting the growth of forb and woody plant species (Erpenbach et al. 2013). Termite mounds can also be used by other non-plant organisms as microhabitats in an ecosystem (Fleming and Loveridge 2003, Costa et al. 2009). Some examples of non-plant organisms include other invertebrates in the Arachnida and Arthropod classes and vertebrate mammal, reptile, and amphibian species (Fleming and Loveridge 2003, Costa et al. 2009).

Nutrient hotspots are created through two main ecosystem processes that are performed by termites: decomposition and bioturbation, which are two processes that have a substantial impact on ecosystem function (Meysman et al. 2006, Lavelle and Spain 2001, Jouquet et al. 2016). Bioturbation is defined as the mixing of soil by living organisms (Meysman et al. 2006). It is an important process in the soil system because it regulates infiltration and diffusion of water, the quality of runoff water, soil erosion resistance, and nutrient cycling that are all essential ecological functions (Jones et al. 2006, Ali et al. 2013). In numerous tropical ecosystems, macroinvertebrates such as termites and ants perform bioturbation (Lavelle 1997, Bignell 2006, Jouquet et al. 2016).

Termites mix and translocate soil aggregates in the creation of their epigeal structures (e.g., casts, sheeting, galleries, and mounds) that they use to establish humid microclimates to avoid desiccation (Jouquet et al. 2011, Bottinelli et al. 2015). As bioturbation is performed by these soil macroinvertebrates, there are indirect consequences produced such as the alteration of the physical and chemical properties of the soil (Frouz and Jilková 2008, Ahmed and Pradhan 2018). When soil is translocated through bioturbation it is loosened and there is a decrease in the bulk density (Frouz and Jilková 2008, Ahmed and Pradhan 2018). An increase in the porous space between soil grains causes changes in the aeration and then indirectly the hydraulic properties of the soil, such as increasing water infiltration rates (Ahmed and Pradhan 2018). Termites build extensive below-ground tunnel and gallery systems when foraging (Marquart et al. 2020). These structures create soil macropores that persist for many years and cause an increase in water infiltration into the soil (Kaiser et al. 2017, Jouquet et al. 2018). The water infiltration rates can increase from a factor of 1 to 4 depending on the nature of rainfall events, the type of soil, and the species of termite that created the macropores (Kaiser et al. 2017).

Bioturbation by termites can also aid in soil profile formation as termites have been shown to favour finer soil grains in the selection for their epigeal structures (Jouquet et al. 2005, Dira and Daniels 2018). The finer soil grains selected by termites are used to build their mounds and differs from the coarser mixture of soil grains found in the rest of the savanna (Jouquet et al. 2005). As a result of this grain size preference, termites can also select for specific indicators (e.g., kimberlite indicators) and soil types, such as clay soils for fungus-growing termites (Jouquet et al. 2005, Dira and Daniels 2018). More indirect consequences of this specificity to soil grain sizes are not only the changes in the soil physical structures but chemical changes that can also occur in the soil bioturbated by termites (de Bruyn and Conacher 1990, Jouquet et al. 2005, Frouz and Jilková 2008). The addition of organic matter into the soil through decomposition as well as the decrease in soil bulk density stimulates microbial activity in the soil which causes an enrichment in mineral nutrients (e.g., NO_3^- and NH_4^+) and exchangeable cations (e.g., Na^+, K^+, Mg_2^+ and Ca_2^+; Jouquet et al. 2004). This enrichment promotes an overall increase in soil fertility and quality which is important in savanna ecosystems that have leached and nutrient-poor soils (Jouquet et al. 2004, Major et al. 2012).

Ants are also considered ecosystem engineers as they influence ecosystem function and structure through processes that regulate ecosystem function and habitat for other organisms (Lavelle 1997, Frouz and Jilková 2008). Ants are an integral part of returning organic matter content into the soil through mixing the soil and performing bioturbation (Del Toro et al. 2012, Wills and Landis 2018, Viles et al. 2021). The construction and maintenance of ant nests also affects physical properties of soil such as the porosity and structure (Frouz and Jilková 2008, Viles et al. 2021). Ant activities (e.g., nest construction) influence the formation of soil aggregates and increase porosity that leads to better water retention and promotion of plant growth (Lavelle et al. 2006, Del Toro et al. 2012). Therefore, changes in soil physical properties that are brought on by ants can result in more productive soils (Del Toro et al. 2012).

Ants are also important predators of termites (Souza and Moura 2008, Lima Pequeno and Pantoja 2012, Fayle et al. 2015). Some ant species, such as *Megaponera analis*, a sub-Saharan African ant also found in savannas, actively scout for termites, and use attack strategies to invade colonies when they are vulnerable or foraging (Yusuf et al. 2014a, 2014b, Frank and Linsenmair 2017). Although ants are important predators of termites, there is a lack of research done at large enough scales to assess the influence ants have on limiting the populations and activity of termites (Parr et al. 2016, Walker et al. 2022). On the same study site used in our study, Parr et al. (2016) and Walker et al. (2022) previously conducted studies where they found that with the reduced abundance of ants there were top-down effects on decomposition and herbivory. Therefore, the relative abundance of ants in ecosystems may influence termite activity through predation. As Walker et al. (2022) observed a top-down effect on decomposition, another important ecosystem process, when ants were removed from the system, there can be an effect on the bioturbation activity of termites in areas where ant abundances are reduced.

The overall aim of this study was to investigate the effect ants and termites have on the bioturbation of savanna soils in the upper 30 cm layer of the soil profile where their activity is greatest. This was achieved by observing the vertical and lateral movement of a blue optical tracer sand and establishing if there was (1) a difference in the amount of optical tracer found when either ants or termites were suppressed and (2) by observing the movement of this tracer between different depth classes over time. The suppression approach conducted by Parr et al. (2016) and Walker et al. (2022) was used in this study. As established in Chapter 2, termites are important bioturbators that move large quantities of soil in a savanna ecosystem. As important termite predators, we predict that the suppression of ants will increase termite activity and result in larger amounts of soil movement, which will in turn increase the amount of the tracer that moves through the soil profile relative to the control. In contrast, the suppression of termites will result in less biotic activity in the soil and as a result, the amount of tracer that will move through the soil profile will be less than in the control and where ants are suppressed.

MATERIALS AND METHODS

Study site

Experiments were undertaken at the Wits Rural Facility (WRF, 24.5667º S, 31.0985º E), located in the Limpopo province, South Africa. The elevation of WRF is ~ 580 m and covers an area of 350 ha of lowveld savanna with nutrient-poor sandy soils. The lowveld savanna is dominated by tree species including *Combretum collinum*, *Dichrostachys cinerea*, *Sclerocarya birrea*, and *Terminalia sericea* (Parr et al. 2016). The grass layer is predominantly *Heteropogon contortus*, *Hyparrhenia* spp., *Panicum maximum*, and *Pogonathria squarrosa* (Parr et al. 2016). The area receives a mean annual rainfall of between 648 ± 194 mm (1992 – 2022) occurring mostly during the summer months. The dry season occurs between May and October in this region.

In 2016 and 2017, a total of 12 experimental plots were established at WRF. In four plots we suppressed the abundance of ants (see Walker et al. 2022) and, in another four plots we suppressed the abundance of termites. The final four plots were designated as control plots (representing natural conditions). The four control plots were 0.25 ha (50 m x 50 m) in size, and each of the suppressed plots was 1 ha (100 m x 100 m) with sampling restricted to the centre 0.25 ha; the buffer zone serves to reduce ants and termites from outside the suppressed plots foraging within the central plot. The plots are 500 m apart. Detailed information on suppression methods is outlined in the supplementary material under “Abundance suppression”.

Soil movement experimental design

In late November 2019, 12 spatial pseudo-replicates, each consisting of two plastic collars (6 cm length cut from 11 cm diameter PVC piping), were placed 5 m apart in a grid pattern per plot (see supplementary material Fig. S1). To ensure no movement of the plastic collars occurred over the course of the experiment, each plastic collar was hammered 4 cm into the surface of the ground. An amount of 65 g of blue coloured sand was spread across the soil surface in each collar; this acted as an optical tracer. The chemicals used to colour the sand were non-toxic. Optical tracers have been used extensively in aquatic ecosystems (Solan et al. 2004, Maire et al. 2008) to observe the movement of sediments but there is not much use of them in terrestrial ecosystems. Elevated plastic basins (42 cm diameter) were inverted and suspended on three metal stakes at 3 cm above the soil surface over each replicate to protect the experiment from heavy rain which could wash the sand away. The basins were also elevated to avoid the creation of a different microclimate underneath them.

Soil movement sampling procedure

The first sampling occasion occurred in late January 2020 (Week 8) and the second occurred in mid July 2020 (Week 32). The study made use of sacrificial samples where one of the two plastic collars was randomly selected for sampling on each occasion (8 and 32 weeks). On each sampling occasion, soil cores were taken from the centre of the plastic collar, and at a 10 cm distance from the collar (lateral cores) using a soil auger (1 cm diameter). The soil auger reached 30 cm into the soil profile. Three segments at 0 – 10 cm, 10 – 20 cm and 20 – 30 cm into the soil profile were then obtained from the soil core and stored as separate samples for later analysis. The lateral core samples were taken diagonally next to the chosen plastic collar to ensure sampling for these cores took place in a different area on each sampling occasion. The upper 30 cm layer of the soil profile was considered as this is where most of the biological activity occurs (Nair 2007, Alamu and Ewete 2021).

Soil core sample analysis

To assess the lateral and vertical movement of the soil over time, the number of grains of tracer sand found at different depths in the soil profile were manually counted. Two homogenised subsamples of 0.75 g each were extracted from each 10 cm segment of soil in a core. Homogenisation was achieved by shaking the sample before the subsample was removed. The subsamples were then placed in a petri dish and the number of grains of blue tracer sand per subsample were counted manually using a microscope and a tally counter. For the vertical movement of the tracer, the 0 – 10 cm soil core in the plastic collar was excluded because of the substantial number of grains of tracer sand that were found on the soil surface (> 2000 grains). A total of 1080 samples were obtained for this study. Half of the samples (n = 540) were taken after eight weeks and the other half after 32 weeks.

Interpretation of tracer movement

Fig. 1 illustrates how the tracer is expected to move through the soil profile. The difference in tracer movement between treatments should be interpreted in relation to depth classes over time (Fig. 1). When there is a small amount of bioturbation the tracer is not dispersed at depth in the profile (Fig. 1a) but when there is a larger amount of bioturbation (and over time) then the tracer is dispersed further in the profile, both laterally and to a greater depth (Fig. 1b and 1c).

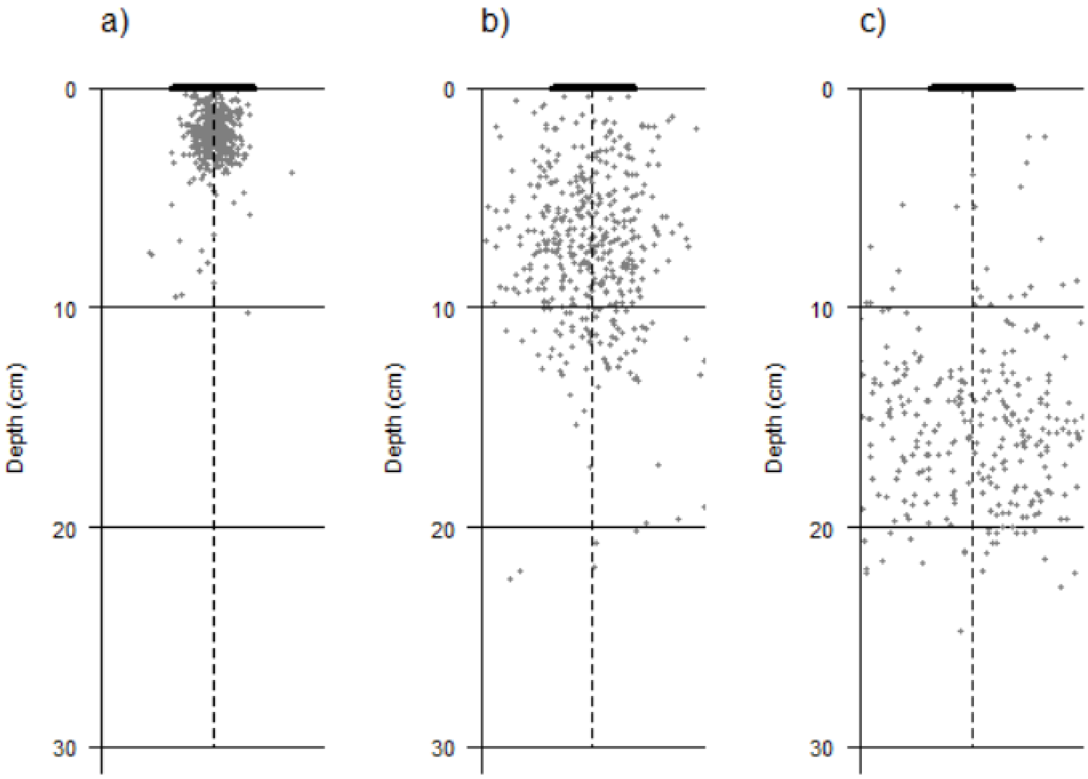

Figure 1: A theoretical diagram depicting how the tracer sand grains are predicted to move vertically in the soil profile through three depth classes (0 – 10 cm, 10 – 20 cm, and 20 – 30 cm) over time. The thick lines at the top of each column symbolise the placement of each plastic collar on the ground surface.

Soil macrofauna

In early February 2020, a total of 15 soil monoliths (length: 20 cm, breadth: 20 cm, height: 20 cm) per plot were sampled to determine if the suppression procedures had any effect on other soil macroinvertebrate species other than the intended ant or termite species. White plastic trays were used for ease of visualisation of macroinvertebrates in the soil and to avoid any loss of soil from the soil monoliths in the search process. An exhaustive search process was conducted until all soil macroinvertebrates were removed from the soil monoliths. Specimens were stored in 70% ethanol and then identified to order level.

Statistical analyses

The difference in the amount of tracer between suppression treatments and the control indicated the effect that suppressed ant or termite abundances had on the process of bioturbation over time. Data for the 12 pseudo-replicates on each treatment plot were pooled so that there was a single value for each of the two depth classes for the core sampled in the collar (10 – 20 cm and 20 – 30 cm) and three depth classes for the core sampled 10 cm away from the collar (0 – 10 cm, 10 – 20 cm, and 20 – 30 cm). Pooling the data removed the presence of pseudo-replication and zero-inflation. Generalised linear mixed-effects models were considered for the statistical analysis but were not used. As the experiment used in this study follows the classical split-plot experimental design outlined in Schielzeth and Nakagawa (2012) where the blocking factor are the four different plots associated with each treatment. As the data has been pooled to include only one observation in each depth class per treatment plots (blocking factor), the potential variance created by the various plots per treatment will be included with the residual variance (Schielzeth and Nakagawa 2012). Therefore, the four different plots associated with each treatment do not need to be included in a model as a random effect or an observation-level-random-effect (OLRE). The location of all the treatment plots at WRF and the consideration of each sampling occasion (week 8 and week 32) separately allowed independence to be maintained. Only two soil depth classes were processed in the core in the plastic collar (Set 1: 10 – 20 cm, 20 – 30 cm) versus three depth classes in the core at 10 cm away from the collar (Set 2: 0 – 10 cm, 10 – 20 cm, 20 – 30 cm) which created unbalanced sampling. To account for this, the data from inside the plastic collar was analysed separately from the data taken 10 cm away from the collar.

For the above reasons, four negative binomial generalised linear models (GLMs) were generated to assess the effect that either ant or termite suppression treatments applied had on the movement of soil over time. The data for all four models suffered from high overdispersion and large outliers. Negative binomial models were chosen because they perform well at high magnitudes of overdispersion ($1.20 \leq$ overdispersion parameter ≤ 1.50) and under outlier dependent scenarios (Payne et al. 2018). When assessing model performance, a model was only accepted if the dispersion parameter fell within these limits. All four of the models used in this study met these criteria. In all four GLMs the number of tracer grains was the response variable, while the treatment applied and the depth in the soil profile as well as the interaction between them were explanatory variables. The interaction between the treatment applied and the depth in the soil profile was only included in models where it had a significant effect ($p < 0.05$). The first model consisted of data for the number of tracer grains counted at 8 weeks in the core sampled in the collar. The second model included data for the number of tracer grains counted at 32 weeks in the core sampled in the collar. The third model consisted of data for the number of tracer grains counted at 8 weeks in the core sampled 10 cm away from the collar. The fourth model included data for the number of tracer grains counted at 32 weeks in the core sampled 10 cm away from the collar. All the models were run in R using the 'MASS' package (Venables and Ripley 2002, R Core Team 2022).

An analysis of similarity (ANOSIM) was conducted to assess whether there was a difference in community composition of macroinvertebrates between the control and suppression treatment plots. The ANOSIM was created using the ‘Vegan’ package in R (Oksanen et al. 2013; R Core Team 2022) and was based on a Bray-Curtis similarity matrix. R-statistic values that are close to one indicate large differences in similarity of the invertebrate community between treatment and control plots. All analyses were performed in R Statistical Software (v4.2.0, R Core Team 2022).

RESULTS

Treatment effect on bioturbation

The quantity of tracer sand grains varied significantly by depth in the soil profile and by the treatment applied for both the 8- and 32-week sampling occasions (Table 1). There was a significant interaction between depth and the treatment applied after 8 weeks in the core sampled in the collar. After 32 weeks in the collar, the quantity of tracer grains did not differ significantly by treatment, but there was a significant difference by depth (Table 1). After 8- and 32-weeks in the core sampled 10 cm away from the collar, the quantity of tracer grains differed significantly by depth and the treatment applied (see supplementary material Table S1 for the models including the insignificant interactions).

Table 1: Summaries for the four negative binomial generalised linear models generated for the number of grains of tracer sand found in the core sampled in the collar (n = 24) and in the core sampled 10 cm away from the collar (n = 36) after 8- and 32- weeks. For each sampling occasion, the number of tracer grains was pooled in each depth class per plot. Reported: the chi-squared statistic (X^2), degrees of freedom (d.f.), and p-values for treatment, depth, and the interaction between the two variables.

Week	**Model**	**X^2**	**d.f.**	**p-value**
Week 8	**Grains in collar ~**			
	Treatment	11.434	2	0.003
	Depth	145.623	1	< 0.001
	Treatment * Depth	8.731	2	0.013
Week 32	**Grains in collar ~**			
	Treatment	5.903	2	0.052
	Depth	136.999	1	< 0.001
Week 8	**Grains 10 cm away from collar ~**			
	Treatment	7.654	2	0.022
	Depth	88.642	2	< 0.001
Week 32	**Grains 10 cm away from collar ~**			
	Treatment	33.270	2	< 0.001
	Depth	20.081	2	< 0.001

Vertical movement

The effect of treatment

The presence of the tracer at lower depths in the soil core that was sampled within the collar indicates that vertical movement of the tracer took place in the soil profile**.** After 8 weeks, there was no significant difference between any of the treatments in the 10 – 20 cm depth class (Fig. 2), but the ant-suppressed treatment had a significantly larger amount of tracer than the termite-suppressed treatment ($p = 0.025$) and the control ($p < 0.001$) in the 20 – 30 cm depth class. There was no significant difference in the amount of tracer between the termite-suppressed treatment and the control after 8 weeks in the 20 – 30 cm depth class. After 32 weeks, there were no significant differences between treatments in the amount of tracer in either of the two depth classes (Fig. 2).

The tracer movement in the soil profile

After 8- and 32- weeks, there was a significantly larger amount of tracer found in the 10 – 20 cm depth class than in the 20 – 30 cm depth class across treatments (Table 2).

As a consequence of not knowing the tracer amount in the 0 – 10 cm depth class in this core, after 8- and 32-weeks the pattern of tracer movement could have been similar to that shown in either Fig. 1b or 1c, where the tracer had moved vertically through the 0 – 10 cm depth class into the 10 – 20 cm depth class and was still moving into the 20 – 30 cm depth class. Although the pattern of tracer movement was similar across all the treatments, a larger amount of the tracer moved into the 20 – 30 cm depth class in the ant-suppressed treatment than in the termite-suppressed treatment and control after 8 weeks.

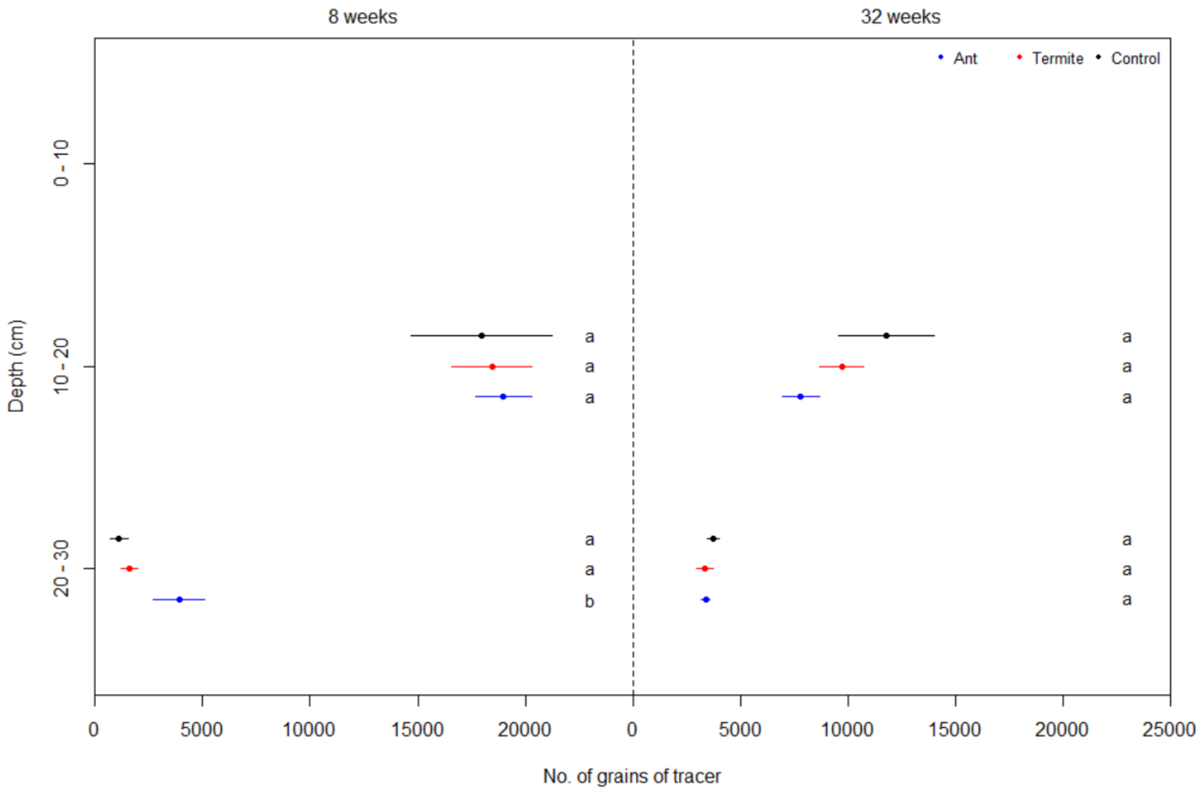

Figure 2: The mean number of grains of tracer sand and standard errors found in two depth classes (10 – 20 cm and 20 – 30 cm) in the soil profile in the collar after 8- and 32- weeks (n = 24) in the control, termite-suppressed (Termite) and ant-suppressed (Ant) treatments. No samples were processed in the 0 – 10 cm depth class in the collar. Different letters among treatments (Ant, Termite or Control) within a depth class and sampling occasion (8 or 32 weeks) indicate significant differences among treatments ($p < 0.05$) for that depth class and sampling occasion.

Table 2: The significant difference in the amount of tracer found in the 10 – 20 cm than in the 20 – 30 cm depth class in the control, termite-suppressed (Termite) and ant-suppressed (Ant) treatments after 8- and 32- weeks for the core sampled in the collar. No samples were processed in the 0 – 10 cm depth class in the collar.

Week	Treatment	Depth Class Contrast	p-value
Week 8	Control	10 – 20 cm vs. 20 – 30 cm	< 0.001
	Termite	10 – 20 cm vs. 20 – 30 cm	< 0.001
	Ant	10 – 20 cm vs. 20 – 30 cm	< 0.001
Week 32	Control	10 – 20 cm vs. 20 – 30 cm	< 0.001
	Termite	10 – 20 cm vs. 20 – 30 cm	< 0.001
	Ant	10 – 20 cm vs. 20 – 30 cm	< 0.001

Lateral movement

The effect of treatment

The presence of the tracer in the soil core that was sampled 10 cm away from the collar indicates that lateral movement of the tracer took place in the soil profile. The smaller amount of tracer in these samples (10 cm from the collar, Fig. 3) indicates that there was less lateral movement of the tracer than vertical movement below the collar (Fig. 2).

After 8 weeks, there was no significant difference between the treatments in all three depth classes (Fig. 3). After 32 weeks, there was significantly less tracer in the termite-suppressed treatment than in the ant-suppressed treatment ($p < 0.001$) and control ($p = 0.002$) for all three depth classes. There was no significant difference between the ant-suppressed treatment and the control in all three depth classes after 32 weeks (Fig. 3). The standard error bars were much larger for the ant-suppressed treatment than for the other treatment and control which indicates that there was high variability in tracer movement among the four ant-suppressed plots (Fig. 3).

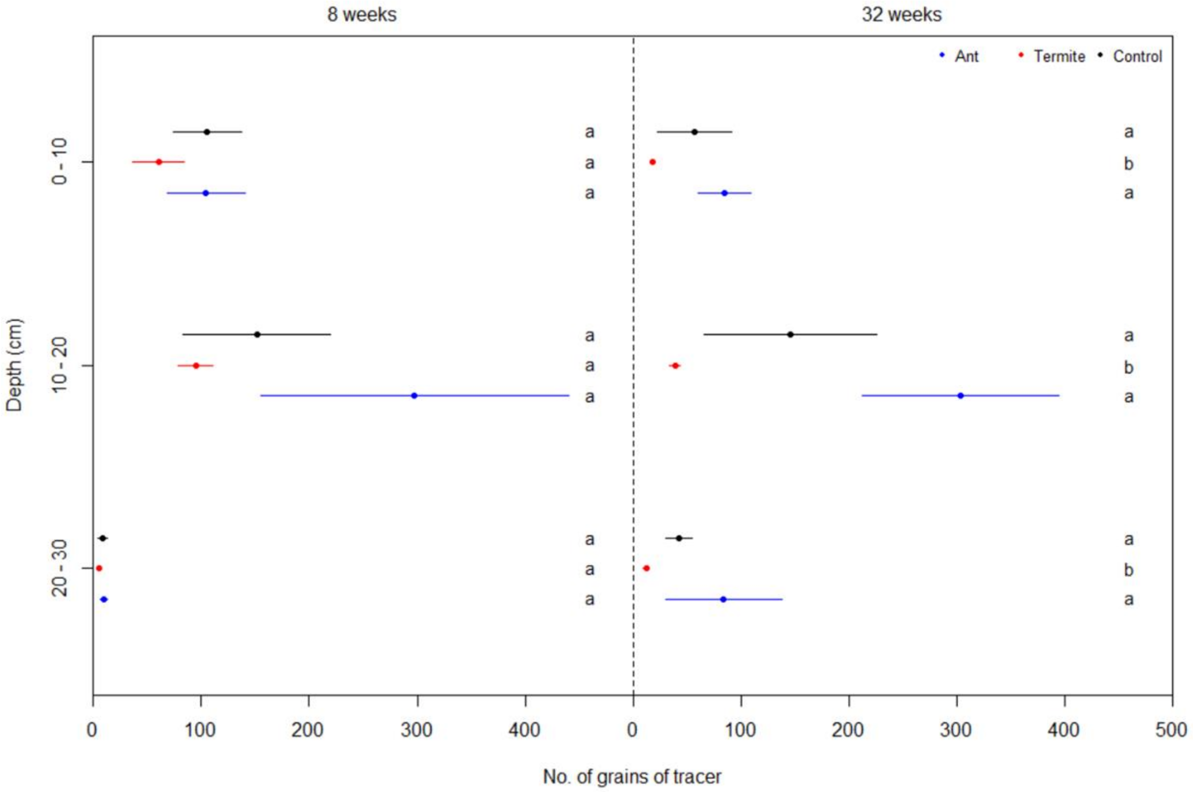

Figure 3: The mean number of grains of tracer sand and standard errors found in three depth classes (0 – 10 cm, 10 – 20 cm, and 20 – 30 cm) in the soil profile sampled at 10 cm away from the collar after 8- and 32- weeks (n = 36) in the control, termite-suppressed (Termite) and ant-suppressed (Ant) treatments. Different letters among treatments (Ant, Termite or Control) within a depth class and sampling occasion (8 or 32 weeks) indicate significant differences among treatments ($p < 0.05$) for that depth class and sampling occasion.

The tracer movement in the soil profile

After 8 weeks, there was a significantly smaller amount of tracer in the 20 – 30 cm depth class than in both the 0 – 10 cm and 10 – 20 cm depth classes across all the treatments (Table 3). There was no significant difference in the amount of tracer between the 0 – 10 cm and the 10 – 20 cm depth classes across all the treatments after 8 weeks. However, after 32 weeks, there was a significantly larger amount of tracer found in the 10 – 20 cm depth class than in the class above (0 – 10 cm) or below (20 – 30 cm) this depth class (Table 3). There was no significant difference in the amount of tracer found in the 0 – 10 cm and 20 – 30 cm depth classes after 32 weeks.

After 8 weeks, the pattern of tracer movement was similar to that shown in Fig. 1b where the tracer had moved through the two shallower depth classes (0 – 10 cm and 10 – 20 cm) and the tracer was only starting to move into the deepest (20 – 30 cm) depth class across all treatments. After 32 weeks, the pattern of tracer movement was similar to that shown in Fig. 1c across treatments. Although the pattern of tracer movement was similar across all the treatments, a smaller amount of tracer was found in all three depth classes in the termite-suppressed plots than in the ant-suppressed and control plots after 32 weeks.

Table 3: The significant difference in the amount of tracer found between the three depth classes (0 - 10 cm, 10 – 20 cm and 20 – 30 cm) in the control, termite-suppressed (Termite) and ant-suppressed (Ant) treatments after 8- and 32- weeks for the core that was sampled 10 cm away from the collar.

Week	**Treatment**	**Depth Class Contrast**	**p-value**
Week 8	Control	0 - 10 cm vs. 10 – 20 cm	0.354
		10 – 20 cm vs. 20 – 30 cm	< 0.001
		0 – 10 cm vs. 20 – 30 cm	< 0.001
	Termite	0 - 10 cm vs. 10 – 20 cm	0.354
		10 – 20 cm vs. 20 – 30 cm	< 0.001
		0 – 10 cm vs. 20 – 30 cm	< 0.001
	Ant	0 - 10 cm vs. 10 – 20 cm	0.354
		10 – 20 cm vs. 20 – 30 cm	< 0.001
		0 – 10 cm vs. 20 – 30 cm	< 0.001
Week 32	Control	0 - 10 cm vs. 10 – 20 cm	0.027
		10 – 20 cm vs. 20 – 30 cm	0.001
		0 – 10 cm vs. 20 – 30 cm	0.997
	Termite	0 - 10 cm vs. 10 – 20 cm	0.027
		10 – 20 cm vs. 20 – 30 cm	0.001
		0 – 10 cm vs. 20 – 30 cm	0.997
	Ant	0 - 10 cm vs. 10 – 20 cm	0.027
		10 – 20 cm vs. 20 – 30 cm	0.001
		0 – 10 cm vs. 20 – 30 cm	0.997

Soil macrofauna composition

The result of the ANOSIM indicated no significant difference in soil macrofauna community composition between the natural (control) and suppression treatment plots (R = 0.004, p = 0.478; Fig. 4).

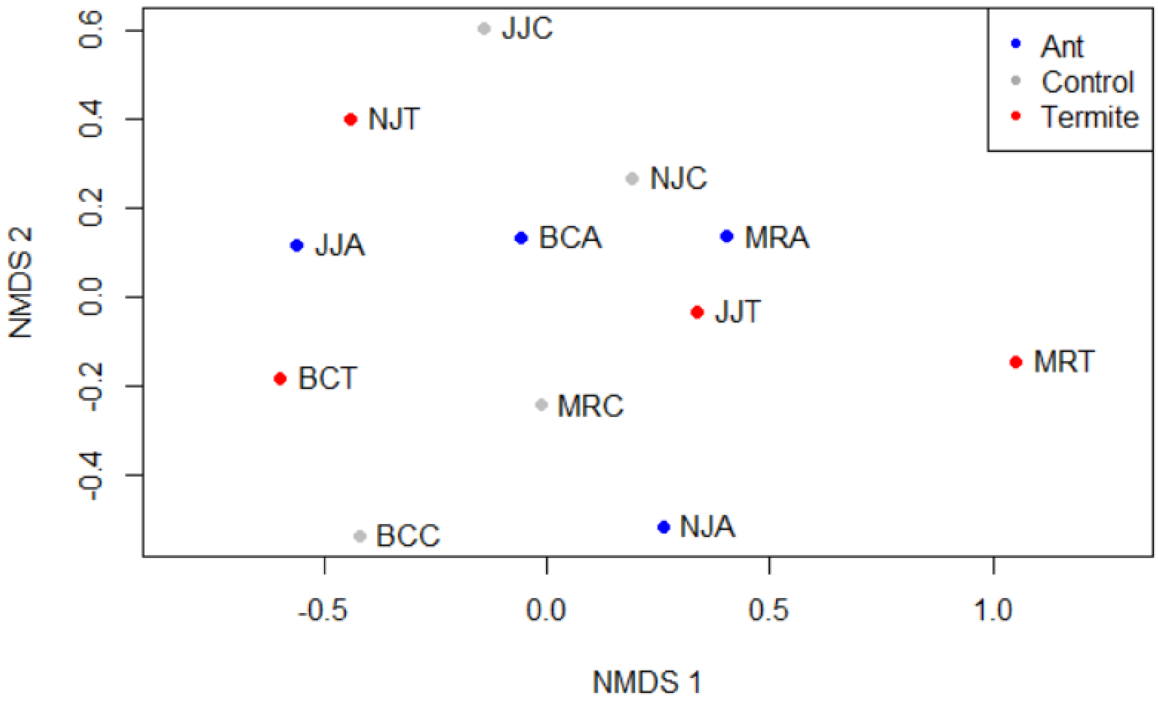

Figure 4: A non-metric multidimensional scaling (MDS) plot visualising the differences in soil macrofauna community compositions among the 12 plots used in this experiment. Four control plots (NJC, JJC, BCC, and MRC), four ant-suppressed (Ant) plots (NJA, JJA, BCA, and MRA) and four termite-suppressed (Termite) plots (NJT, JJT, BCT, and MRT).

DISCUSSION

In this study we investigated to what extent ants and termites influence bioturbation in savanna soils. This was achieved by suppressing ant and termite abundances and then observing the amount of an optical tracer sand that was counted at different depth classes in the soil profile as a proxy for the process of bioturbation. We predicted that decreased ant abundance would result in more termite-mediated bioturbation relative to natural conditions because of a top-down control of termites via ant predation. We also predicted that because bioturbation was mainly termite-mediated, a reduction in their abundance would result in less bioturbation relative to natural abundance conditions.

In agreement with our predictions, significantly larger amounts of tracer were recorded in the deepest depth class in the soil profile on the ant-suppressed treatment after 8 weeks (in the collar). The increase in bioturbation cannot be attributed to the presence of other vertebrates and invertebrates that move soil to obtain subterranean resources (e.g., water and food, and building shelters or nests) as their presence and influence on soil movement on the plots was not affected by the suppression treatments. Therefore, the reduction of ant abundance, achieved by suppression methods, increased the amount of termite-mediated bioturbation after 8 weeks. Termites are important bioturbators because they perform bioturbation at a large scale when creating their epigeal structures and by foraging (Wilkinson et al. 2009). Lateral and vertical movement of soil occurs when termites are constructing their epigeal structures (Jouquet et al. 2011). It has been reported that especially in the subfamily Macrotermitinae and the genus *Trinervitermes*, large quantities of soil can be moved from various depths in the soil profile during construction (Holt and Lepage 2000, Brossard et al. 2007). Termite foraging activities can occur up to a depth of 40 cm below the surface of soil (Nair 2007, Alamu and Ewete 2021). The mixing of soil during these activities can process between 3- to- 53 tons ha^{-1}. y^{-1} of soil in temperate regions and 730- to- 1100- ton ha^{-1}. y^{-1} in tropical regions (Wilkinson et al. 2009). However, the ramifications for bioturbation caused by ant top-down control have not been explored until now.

Ants are one of the most important predators of termites (Dambros et al. 2016, Tuma et al. 2020, Basset et al. 2020). Most ant species are generalist predators, but the presence of various extensive defence mechanisms that termites have to reduce predation by ants indicates the significant impact that ant predation has had on the selective pressure of termites (Eggleton 2010, Tuma et al. 2020). It is highly likely that the increase in termite-mediated bioturbation was caused by the reduction of ant top-down control on termites because the effects of other animal groups (e.g., pangolins and aardvarks) that are also known predators of termites would be the same across all treatments and was therefore negligible (Swart et al. 1999, Taylor et al. 2002, Tuma et al. 2020). In contrast to these animal groups, ants are more widespread spatially and are major predators of termites that are both actively foraging and located in their mounds (Hölldobler and Wilson 1990) to the degree that ant density and richness has even been related to termite density in other ecosystems (Leponce et al. 1999, Mertl et al. 2012). Leponce et al. (1999) found that the presence and establishment of termite nests can depend on the presence of already established ant predator species nests indicating termite avoidance of ant predation pressure.

Furthermore, we observed significantly less tracer across all depth classes sampled in the soil profile in the termite-suppressed treatment in relation to the control or ant-suppressed treatments after 32 weeks (at 10 cm away from the collar). This is an indication that less bioturbation occurs when termite abundance is low and highlights their role as important bioturbators.

Collectively, these results show that the manipulation of ant and termite abundances has an impact on the movement of savanna soils and reveals the importance of these macroinvertebrates in the important ecosystem process of bioturbation. The soil pits, that were sampled at 9 weeks into the experiment (February 2020), also indicated that there were no significant treatment-level differences in the soil macroinvertebrate community structure, confirming that the chemical suppression treatments did not have an influence on the invertebrate community in the soil.

Soil Movement and Sampling Timing

Accurately quantifying the process of bioturbation using this optical tracer technique is dependent on the time at which samples are taken in the soil profile in relation to the addition of the tracer to the soil surface. There is likely to be a time-window within which treatment-level effects can be detected and this may differ with sampling position in relation to the tracer source (e.g., in the collar versus 10 cm away from the collar). If sampling takes place too soon after the tracer is placed on the soil surface, then no treatment effects are likely to be observed because there is insufficient time for soil to be dispersed through the profile. Conversely, if the time-period is too long then all of the tracer may have already moved through the profile and dispersed. Significant treatment-level effects were detected vertically (in the collar) after 8 weeks but these effects were no longer detectable by 32 weeks. This is likely due to two reasons. Firstly, the sampling interval was likely too long to detect these effects. Secondly, the long sampling interval meant that sampling took place during the dry season, when termite and ant activity was lower than activity in the wet season (Dawes-Gromadzki 2003, Davies et al. 2015), resulting in lower between-treatment effects of ant and termite suppression. The intended plan of sampling at a shorter interval at 16 weeks could not take place due to COVID-19 regulations.

At 10 cm away from the collar no lateral treatment effects were detected after 8 weeks, but significant effects were detected after 32 weeks. When termites mix soil, they translocate it vertically and laterally from multiple depths in the soil profile (de Bruyn and Conacher 1990, Jouquet et al. 2011). The presence of the tracer 10 cm away from the collar confirms that lateral movement occurred but the smaller amount of tracer here suggests that there was less lateral than vertical movement of soil or that lateral movement is potentially slower than vertical movement. Therefore, 8 weeks may have been too short a time-period to observe treatment effects at this distance away from the collar.

Limitations and refinements

The confounding seasonal effect on termite and ant activity that was introduced because sampling took place at 32 weeks (instead of the planned 16 weeks) is a limitation to the study. The effects of suppression of either ant or termite abundance would have had less effect during the dry season because of the lowered activity of ants and termites (Dawes-Gromadzki 2003, Davies et al. 2015). Even though termite abundance and activity are likely to have increased because of ant suppression, reduced termite activity in the dry season likely caused less termite-mediated bioturbation on ant-suppressed plots. Ant predation pressure could also be reduced in the dry season, resulting in a less pronounced effect of ant-suppression at this time.

Treatment-level effects may have been easier to detect with a greater number of replicates (plots), however logistical constraints because of the large plot size (1 ha) precluded additional replicates. The PVC piping used to contain the optical tracer may have obstructed the lateral movement of the tracer in the top 0 - 10 cm depth class because it was hammered 4 cm into the soil. Although obstruction of lateral tracer movement in the 0 – 10 cm depth class could have been a limitation, the PVC piping was necessary to contain the tracer on the soil surface. Lastly, 10 cm depth classes were used for quantifying the amount of tracer in the soil profile. A depth class as wide as 10 cm is more likely to mask subtle differences in the amount of tracer among treatments than a narrower depth class (e.g., 1 cm). Future studies could take samples at 5 - 6 cm, 15 - 16 cm, and 25 - 26 cm, which would likely improve the sensitivity of the technique.

CONCLUSION

This study shows that termites and ants influenced the movement of soil in a savanna. Our study is the first to demonstrate that termite-mediated bioturbation in the soil profile can be affected by top-down control via ant-predation at a large scale in a natural setting. The suppression of ant abundance caused more soil movement which could be attributed to the reduction of predation pressure, thus facilitating increased termite-mediated bioturbation. The suppression of termite abundance caused less bioturbation to occur than that occurring in natural conditions. Our study highlights the importance of termites as bioturbators in an African savanna ecosystem and how the interaction between them and their most influential predator, ants, can influence an important ecosystem process such as bioturbation. It also adds to recent work that suggests that top-down control by ants as predators can influence termite-mediated decomposition, another important ecosystem process (Walker et al. 2022). Understanding the interaction between ants and termites and how it affects the surrounding landscape can be important in a future with climate change and further habitat transformation and fragmentation. Ants and other predators will likely experience declines in diversity under climate change conditions (Wilmers et al. 2007, Bertelsmeier et al. 2016, Laws 2017). Therefore, understanding how these two macroinvertebrate taxa influence each other is essential for understanding ecosystem functioning and future habitat restoration schemes (Wijas and Atkinson 2021).

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CHAPTER 2: SUPPLEMENTARY MATERIAL

Soil movement experimental design

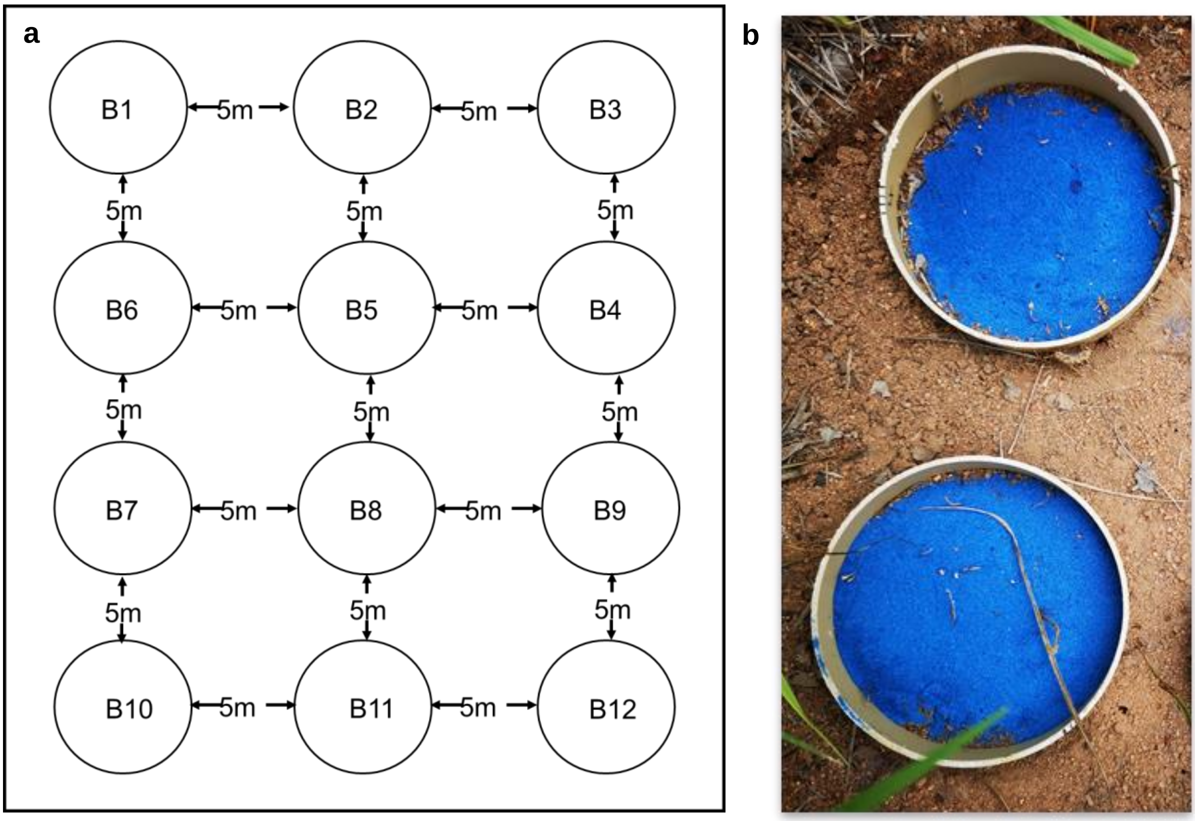

Figure S1: The experimental layout that occurred on each plot. **a)** The spatial arrangement of 12 pseudo-replicates of basins (B1 to B12, 42 cm diameter) that were placed 5 m apart and elevated by 3 cm to protect from rain and to prevent the creation of humid microhabitats. **b)** A photograph of the two plastic collars (height = 6 cm, diameter = 11 cm) located under each basin with 65 g of a blue optical tracer sand spread across the soil surface in each collar. A soil core was taken from one of the two collars at 8 weeks and another was taken from the remaining collar at 32 weeks. Soil cores were also taken 10 cm (laterally) from each collar at 8 and 32 weeks.

Treatment effect on bioturbation

Table S1: Summaries for the four negative binomial generalised linear models that would have been generated for the number of grains of tracer sand found in the core sampled in the collar (n = 24) and in the core sampled 10 cm away from the collar (n = 36) after 8- and 32- weeks if insignificant interactions between variables were included. For each sampling occasion, the number of tracer grains was pooled in each depth class per plot. Reported: the chi-squared statistic (X^2), degrees of freedom (d.f.), and p-values for treatment, depth, and the significant or insignificant interaction between the two variables.

Week	**Model**	**X^2**	**d.f.**	**p-value**
Week 8	**Grains in collar ~**			
	Treatment	11.434	2	0.003
	Depth	145.623	1	< 0.001
	Treatment * Depth	8.731	2	0.013
Week 32	**Grains in collar ~**			
	Treatment	6.584	2	0.037
	Depth	152.750	1	< 0.001
	Treatment * Depth	2.800	2	0.247
Week 8	**Grains 10 cm away from collar ~**			
	Treatment	7.960	2	0.019
	Depth	92.154	2	< 0.001
	Treatment * Depth	1.437	4	0.838
Week 32	**Grains 10 cm away from collar ~**			
	Treatment	33.743	2	< 0.001
	Depth	20.368	2	< 0.001
	Treatment * Depth	0.595	4	0.964

Abundance suppression

Two synthetic insecticides were used to suppress termites. The first insecticide, Premise 200SC (Bayer, Environmental Science) that has an active ingredient imidacloprid occurring at 23 ppm (0.00235), was used to suppress mound-building termite species. All existing mounds on the suppression plots were destroyed. The smaller *Nitiditermes* spp. (previously known as *Cubitermes* spp.) mounds were destroyed by hand and the larger *Macrotermes* spp. mounds were destroyed by a tractor-loader-backbone and pickaxes. The exposed soil left over after the mounds were destroyed was then sprayed with imidacloprid solution. *Macrotermes* spp. mounds were also destroyed just outside of the 1 ha suppression plots as their foraging range would extend into the suppression plots. The second insecticide, Agenda 20C (Bayer, Environmental Science) has the active ingredient fipronil occurring at 5.70 ppm, was used to suppress non-mound-building termites and any mound-building termites that possibly survived the previous insecticide. Subterranean termites were poisoned using wood shavings, rooibos tea bags, and toilet paper rolls. The toilet paper rolls were sawn in half and soaked in fipronil, after which they were dried in the shade for 2 - 3 days. There were 441 halved toilet paper rolls placed every 5 m in a 21 x 21 grid on each of the suppression plots and were buried between a 5 – 10 cm depth in the soil. Wood shavings were also soaked for 12 hours in fipronil and placed 5 m apart in a 21 x 21 grid at 5 – 10 cm depths in each suppression plot. The rooibos tea bags, which were also soaked in fipronil for 12 hours were placed every 3.5 m in a 22 x 23 grid across the suppression plots. Application of imidacloprid occurred every 3 months and fipronil every month in the activity season of termites.

Two synthetic insecticides in baits were used on ant-suppression plots (Walker et al. 2022). The first insecticide was Maxforce™ Ant Bait Granules (Bayer, Environmental Science, Isando, South Africa) that contains Hydramethylon at 1% which is mixed into a corn grit and soybean oil bait. The second insecticide was constructed by Parr et al. (2016) and consisted of Maxforce™ that is supplemented with protein-carbohydrates in the form of the Whiskas™ cat food brand, gelatin, sugar, and imidacloprid at 5 ppm all mixed to create a bait. The industrial made Maxforce™ baits (3 kg per plot) and the homemade Maxforce™ baits (6 kg per plot) were applied to the ant-suppressed plots in six parallel lines spaced 5 m apart (Walker et al. 2022).

CHAPTER 4: GENERAL DISCUSSION

RESEARCH OVERVIEW

The two data chapters featured in this thesis presented research outcomes that contribute to the understanding of the role two macroinvertebrate taxa (termites and ants) have in bioturbation in a broad leaf African savanna. I rigorously tested a photogrammetric technique to estimate the amount of soil moved by two termite genera (*Macrotermes* spp. and *Nitiditermes* spp.) when constructing their mounds, to highlight the importance of termites as bioturbators in a savanna ecosystem. I made use of established suppression treatments to study the potential top-down effect that ants, as important predators of termites, would have on termite-mediated bioturbation in a savanna.

CONTRIBUTIONS AND CONSIDERATIONS

My study used photogrammetry to successfully quantify the changes in termite mound volume over time and produced comparably low error values and high precision to Nauer et al. (2018), a previous study that used photogrammetry to estimate the volume of termite mounds in an Australian coastal savanna woodland. Nauer et al. (2018) also calculated the volume of intact termite mounds at a single point in time using SfM photogrammetry. My study is unique as it is the first to use SfM photogrammetry to quantify the temporal change in termite mound volume. The strength of my study is that even with overestimation of mound volumes, it is consistent enough to allow for correction factors to be applied in future studies, and the photogrammetric technique has the potential for broader applications. Photogrammetry is also quick and easy to apply in the field where it took on average 10 – 15 min per mound, dependent on mound size. It takes a similar amount of time to apply more traditional methods (e.g., measuring mound height and circumference) to estimate termite mound volume (Darlington and Dransfield 1987). Therefore, the high precision and quick application in the field highlights photogrammetry as a suitable method to estimate the volume of termite mounds.

The accuracy and precision of photogrammetry is dependent on the camera equipment and software used (de Reu et al. 2013, Koutsoudis et al. 2013, 2014). My validated photogrammetric technique demonstrated that images taken of termite mounds with a single consumer grade DSLR camera can allow for sufficiently detailed 3D models of termite mounds to be produced enabling their volume estimation. A previous study assessing the ecological characteristics of coral suggested that single lens cameras are preferrable when doing an image analysis because of their large sensors that enable better light sensitivity (Burns et al. 2015). Furthermore, Bot and Irschick (2019) stated that using a single camera, as opposed to a set of cameras is best suited when subjects are static (e.g., termite mounds) as it enables easier usage in the field. Nauer et al. (2018) used a mirrorless camera with low-cost and free software packages when using photogrammetry to estimate the volume of termite mounds. My study differs in that it demonstrates that a single consumer grade camera with mirrors can also be used in conjunction with a high-cost software (PhotoModeler ® Premium) to estimate the volume of termite mounds.

As suggested for future studies by Nauer et al. (2018), I used a software that had automated feature detection enabling the boundary between the termite mound and surrounding soil to be more distinct. Harwin and Lucieer (2012) suggested that the density of point clouds that are generated in software, like PhotoModeler ® Premium, are sufficient with consumer grade cameras that are uncalibrated. A calibrated camera (used in this study) should increase the density of point clouds enabling a detailed surface of the subject to be captured at the time of data acquisition. Therefore, increasing the feasibility of the DSMs generated from the high-density point clouds. Eulitz and Reiss (2015) were able to use 3D photogrammetry to construct highly detailed 3D models for scanning electron microscopy (SEM) through the usage of high-density point clouds. Chiari et al. (2008) created a hybrid method of 3D photogrammetry and stereo vision. Stereo vision uses pairs of cameras at a fixed distance apart to generate highly spatial dense points of a subject’s surface (Chiari et al. 2008). The hybrid method employed by Chiari et al. (2008) made use of landmarks that were present in multiple images of the subject, where images were taken at a similar distance apart to the approximate distance of camera pairs in stereo vision. My study made use of high-density point clouds, a large number overlapping images and landmarks (i.e., rulers and triangular markers) that generated 3D models with detail comparable to the models generated from the hybrid method created in Chiari et al. (2008).

Photogrammetry is also non-invasive, which is an improvement over other methods used to measure the volume of termite mounds such as water displacement (Lavy et al. 2015, Bot and Irschick 2019, Olinger et al. 2019). Instead, the termite mounds could be left in the field and the natural addition or erosion of mound soil was observed over time without disturbance. Vegetation around the mounds was also removed in a non-invasive or destructive manner that did not disturb or damage the mounds.

The complexity of termite mounds has made it difficult to quantify basic physical parameters such as volume. However, precise estimates of termite mound physical parameters are important in the studies of termite ecology, biochemistry, and physiology (Nauer et al. 2018). My study represents an example of an ecological application where using photogrammetry can be applied to quantify the bioturbation performed by termites in an ecosystem. It contributes to the understanding of how photogrammetry can be used on termite mounds to estimate physical parameters that are important when assessing the ecological role of termites.

Previously, surface soil deposition rates of burrowing invertebrates have been quantified using indirect estimates (Wilkinson et al. 2009, Bétard 2020). Earthworm bioturbation has been quantified by calculating total earthworm biomass and rates of soil egestion (Taylor et al. 2019). Ant bioturbation has been quantified by multiplying the number of ant nests per hectare by the dry weight of nests and dividing that by nest residence time (Richards et al. 2011, Taylor et al. 2019). The presence and measurement of termite structures such as sheetings is commonly used in quantifying termite bioturbation (Jouquet et al. 2021, Walker et al. 2022). In Namibia on open grassland and acacia savanna, Turner et al. (2006) collected and weighed newly deposited soil on termite mounds to quantify soil transported by termites. Tschinkel (2015) used the lateral spread of pink coloured sand placed on the soil surface to assess how wind, water, and animals can change the size of excavated soil discs that are indicative of the size and depth of harvester ant nests. However, to my knowledge no study has quantified the ecological role termites and ants have via bioturbation in an ecosystem or assessed the effect of their interspecific interaction.

It is well known that interspecific interactions determine community structure (Wang et al. 2021) and change over time (Fox and Morin 2001). However, little is known about how interspecific interactions indirectly control ecosystem functioning through ecological processes like bioturbation. My study provides important insight into how interactions between two macroinvertebrate taxa can potentially alter bioturbation, an essential ecological process. I demonstrate that ecosystem processes can potentially be altered indirectly via a predator-prey interaction. This study is also one of very few (Parr et al. 2016, Walker et al. 2022) conducted using large-scale suppression treatments to evaluate the effects that ants have on termite activity and is the first to directly assess termite-mediated bioturbation in the soil profile. My study is unique in that it is the first to quantify the lateral and vertical movement of an optical tracer at a small scale (10 cm depth classes) in the top 30 cm of the soil profile over time to assess the role that an interspecific interaction has on bioturbation in an ecosystem.

In this thesis, I have demonstrated that termites and ants are important ecosystem engineers in an African savanna. Termites are fundamental to bioturbation in a savanna ecosystem and ants have the potential to exert top-down control on termite-mediated bioturbation as important termite predators. Bioturbation performed by termites when constructing their nests and epigeal structures alters the physical and chemical properties of soil such as hydraulic properties and nutrient concentrations (Jouquet et al. 2011, Li et al. 2017). Termite mounds promote spatial heterogeneity in savannas by being the location of nutrient hotspots that promote the growth of forb and woody plant species (Moe et al. 2009, Erpenbach et al. 2013, Davies et al. 2016). The volume of soil added to termite mounds in this thesis is indicative of the key role they play in nutrient cycling and soil formation in savanna ecosystems. Ants are natural important predators of termites (Tuma et al. 2020). Therefore, ants and their top-down control on termite-mediated bioturbation is an essential aspect of savanna ecosystem function. These findings are highly relevant in the context of ecological functioning under climate change. Climate change is predicted to alter predator-prey interactions (Laws 2017). However, the ability to predict how organisms that have essential ecological roles will respond to climate change and the consequences to the ecosystems of which they are a part of is essential for future conservation and restoration schemes.

SUGGESTIONS FOR FURTHER RESEARCH

This study provided valuable insight into the role that two macroinvertebrate taxa have in bioturbation in an African savanna. Further support for these findings could be provided by future research topics:

i. The artificial mound sizes in my study were logistically limited and not inclusive of the upper range of *Macrotermes* mound volumes assessed in the field. For future validation of photogrammetric techniques, objects (e.g., artificial mounds) should be representative of the full range of termite mound volumes occurring in the field. Other methods of validating the photogrammetric technique could be used, such as laser scanning, as a ground truth measurement to compare with the volume estimations under validation (James and Robson 2012).

ii. Previous studies have demonstrated that 3D photogrammetry can be highly accurate and precise with no over- or under- estimations of subject parameters when generating 3D models of turtles (Irschick et al. 2020a), reptiles and amphibians (Irschick et al. 2020b), and harbor porpoises (Irschick et al. 2020c). These studies made use of other software (e.g., Blender) to generate 3D models. Therefore, the comparison of 3D models and the subsequent volumes of termite mounds generated by other software could be beneficial in future studies to assess the extent of which software used influences model accuracy.

iii. Literature suggests that 3D photogrammetry can be very accurate when avoiding the usage of wide-angle lenses and suggests that lenses from 25 – 55 mm focal length be used (Aldridge et al. 2005, Chiari et al. 2008, Irschick et al. 2020c). A wide-angle lens (18 mm) was used in this study for the inclusion of larger termite mounds. For future studies, smaller termite mounds and different focal lengths (wide versus narrow) could be used to compare between lens focal length and whether distortion form the wide-angle lens created the overestimation observed in this study. Furthermore, focal lengths could be adjusted dependent on the subject as demonstrated by Irschick et al. (2020a) when modelling turtles of differing sizes.

iv. Other applications such as X-ray, computer tomography (CT scanning), have been used to describe the internal structure of termite mounds (Perna et al. 2008, Perna and Theraulaz 2017). The use of CT scanning in future studies will provide further insight into the extent of mound volume that can be attributed to internal structures (i.e., pore space) for more accurate estimations on the weight of soil moved. However, CT scanning requires expensive medical equipment, and its use on termite mounds of differing sizes is limited by the scanner capacity (Nauer et al. 2018). Therefore, feasibility of this application should be considered.

v. The photogrammetric technique used in the current study had an upper limit of *Macrotermes* mound size, and the volume of larger, vegetated mounds could not be estimated. In future studies, photogrammetric techniques that can include these larger mounds would be more beneficial as the consideration of the amount of soil translocated by the large colonies inhabiting these mounds is important in an ecosystem. For inclusion of these mounds unmanned-arial-vehicles (UAVs) in conjunction with light detection and ranging (LiDAR) or SfM photogrammetry techniques could aid in observing the change in volume over time.

vi. For future studies, larger sample sizes of *Macrotermes* mounds would be preferable. A non-linear relationship with growth, such as the sigmoid growth curve seen in trees (Weiner and Thomas 2001) could be present in *Macrotermes* mounds. *Macrotermes* mounds that are several orders of magnitude larger than the largest mounds measured in my study can be observed in the field. However, a larger sample size with greater temporal sampling will provide more information on the relationship between initial mound volume and the subsequent rate of change in volume.

vii. A major limitation in my study was the long interval between sampling periods because of COVID-19 regulations. For chapter 2, the seasonal difference in the rate of change in mound volumes would have benefitted if the wet and dry season were more appropriately subdivided. For chapter 3, treatment-level effects are more likely detected in certain time-windows. If sampling takes place too soon after the optical tracer is placed on the ground surface, then no treatment-level effects are likely to be observed. Conversely, if the time frame between sampling occasions is too long then all of the optical tracer may have already moved through the soil profile to deeper depths (> 30 cm) than sampled. Furthermore, the long time frame between sampling occasions in chapter 3 introduced a confounding effect of seasonality where ant and termite activity will be lower in the dry season. Therefore, future studies should plan appropriate sampling occasion intervals accordingly.

viii. For chapter 2, I suggest that increased temporal measurements (e.g., weekly) of termite mound volumes could also provide vital information on the smaller volume changes that termite mounds undergo in relation to erosion associated with extreme rainfall events. Subsequent 3D models could also be compared to assess the minor changes in volume and external structure of termite mounds. Future studies could also make use of the suppression treatments to assess if there is a difference in mound volume changes when ant abundance is reduced. No mounds were selected on the termite and ant suppression plots in this study as there were either no mounds on the plots because of chemical poisoning (termite suppression) or the mounds found on these plots were too large for the chosen photogrammetry procedure to estimate (ant suppression).

ix. For chapter 3, I suggest that narrower depth classes (e.g., 1 cm) be used as the broader depth class (10 cm) used in my study could be masking more subtle treatment-level differences. Suppression treatment-level effects could also benefit from more replicates (plots). Including plots where both ant and termite abundance have been suppressed could provide further insight into effects on ecosystem processes when both macroinvertebrate taxa are reduced. In my study, for logistical reasons, more replicates, and the suppression of both macroinvertebrates on one plot were not feasible.

CONCLUSIONS

The findings of this thesis provide further insight into the importance of termites as bioturbators and the influence that ant predation has on their activity in a savanna ecosystem. Termites can move between ~ 3 kg to ~ 450 kg of soil over 40 weeks in a savanna through termite-mediated bioturbation when building their mounds. The amount of soil moved is dependent on the termite genus being considered. The weight estimates presented in this thesis are likely underestimates as larger termite mounds, which tend to house larger colonies, are likely to move substantially larger amounts of soil. The suppression of termite abundance causes less bioturbation to occur when compared to natural conditions. However, when suppressing the abundance of ants, the most influential predator of termites, there is a potential for more termite-mediated bioturbation to occur. Therefore, ants have the potential to influence termite-mediated bioturbation through top-down control. Further investigations need to be conducted into the effect that ants have on termite-mediated bioturbation and the consequences for ecosystems where the two macroinvertebrate taxa interact. It can be concluded that the presence or absence of termites and ants can have significant impacts on bioturbation, an essential ecological process, in savanna ecosystems.

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