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Review Article

Advances in Nanoscale Study of Organomineral Complexes of Termite Mounds and Associated Soils: A Systematic Review

Peter N. Eze , Atlasaone Kokwe, and Juliet U. Eze³

- ¹Department of Earth & Environmental Science, Botswana International University of Science & Technology, Private Bag 016, Palapye, Botswana
- ²Department of Environmental Science, University of Botswana, Gaborone, Botswana
- ³Department of Biological Sciences & Biotechnology, Botswana International University of Science & Technology, Palapye, Botswana

Correspondence should be addressed to Peter N. Eze; ezep@biust.ac.bw

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Termite mounds are replete with natural nanoparticles, and they vary in physicochemical, geochemical, mineralogical, and biological properties from the adjoining soils. Although termite mounds have wide ecological and environmental roles including soil formation, faunal and vegetation growth and diversity, organic matter decomposition, geochemical exploration, water survey, treatment of underground contamination, thermoregulation, gas exchange, and global climate change, their nanoscale structures made by the associated organomineral complexes are still poorly understood because of technical limitations. In this review, we highlight the ecological and environmental significance of termite mounds and the documented techniques that have been successfully used to study nanostructure of termite mounds, namely, midinfrared spectroscopy (MIRS), photogrammetry and cross-sectional image analysis, a combination of transmission electron microscopy (TEM) and pyrolysis field ionization mass spectrometry (Py-FIMS), scanning transmission X-ray microscopy (STXM) using synchrotron radiation in conjunction with near-edge X-ray absorption fine structure (NEXAFS) spectroscopy, and high-resolution magic angle spinning nuclear magnetic resonance (HR-MAS NMR) for further appraisals. There is a need to continually develop and integrate nanotechnology with the routine classical soil analysis methods to improve our understanding of the functional mechanisms of nanostructure of termite mounds that are responsible for specific properties. In view of the numerous roles termite mounds play in the environments, agriculture, and engineering, there is no better time to channel much research into understanding how they function at nanoscale.

1. Introduction

A myriad of microbial and animal species live and make up the soils, from bacteria to macro organisms including insects. Termites are amongst the most successful of all soil organisms inhabiting most landmasses except Antarctica [1], with Africa having the largest number of fungus growing termites [2]. Termites occupy about 40–60% biomass of the macro fauna in the tropics [3] and in the African Savannah an estimated biomass of 70–110 kg·ha¹ making them the most abundant macro fauna in the soil. They are generally grouped into (i) epigeal (above ground, usually treedwelling) or hypogeal (below ground), (ii) forage primarily for wood, litter, or humus, (iii) construct distinct nests, and

(iv) cultivate fungi [4]. Termites construct biological structures which serve different roles such as protecting them from predators, environmental hazards, and humidity.

Termites construct mounds by reworking soils, transporting soils below ground to the Earth surface. As soil engineers, they build galleries and termite mounds which may have an impact on the microbial component of the soil [5] as they modify the soil environment and affect other organisms. Their construction techniques differ according to the environmental conditions, species, and their feeding habit. In a study by Jouquet et al. [6] to determine the exact depths at which termites collect soils, they found that cathedral mounds had same properties as Ferralsols at 96 cm and as Vertisols at 49 cm. For lenticular mounds they were

shallower than cathedral at 30 cm for Ferralsols and 64 cm in Vertisols. The soil feeders (Termitinae) build their structures with fecal matter and inorganic material, while fungus-growing termites (Microtermitinae) build by soil and clay and are cemented by salivary excretions. Basically their structures are made by mineral matrix mixed with saliva and faeces (organomineral complexes), adding C and N nutrients. These organomineral nest walls have poor structural stability.

Termite mounds (Figure 1) are found all over the tropics and the subtropics [7, 8], which constitute 1/6 of the Earth [9]. Several studies have been undertaken throughout the world on different termite species mounds and their physicochemical properties, distributions, and management practices (e.g., [10-13]). The last four decades have witnessed a growing trend in publications mainly centred on the roles of termites in ecosystem functioning [14-17]. Besides various ecosystem functions, scientists have explored the roles of termite mounds in other services including gas exchange and thermoregulation, geochemical exploration, biomimicry, and global climate change. Understanding the mechanisms by which termite mounds function and how their specific properties are formed requires an examination of the termite mounds at micro- and nanoscales. Lack of appropriate methodologies to carry out such studies has been of great concern amongst soil ecologists. In this review, we knit together the properties of termite mounds in relation to the surrounding soils and outline the various recent technological advances available for the study of the nanoscale structures of termite mounds that are responsible for their properties.

2. Prominent Mound Building Termite Species in Tropical Environments

About 2600 termite species exist worldwide with the highest termite diversity found in Africa [5]. The most abundant species in Africa is the litter-feeding termites, Macrotermes [13]. This species collects clay particles deep down the soil layers and brings them to the soil surface. Because of the clays, their structures are usually resistant to harsh weather conditions as clay minerals give resistance to the structures by their surface properties such as cohesion and by the cementation of the larger sized particles. These species protect their colonies and have structures that are cathedral and dome-shaped [11]. Central African countries are mainly characterized by the species Macrotermes falciger, which originates from Lubumbashi in the southeastern of Democratic Republic of the Congo. They construct termite mounds that could be as high as 10 meters, the highest mound height ever recorded in all parts of Africa [18]. They are found in woodlands and they are the main biodigesters in this region, hence the remarkable heights. Macrotermes falciger prefer a warm humid climate; therefore they adapt by building closed ventilation structures with thick walls in order to preserve heat for their metabolic activities, while another species of termites, Reticulitermes flavipes (Kollar), becomes inactive in winter, in which no activity takes place [19]; they do not show any evidence of temperature



FIGURE 1: A *Macrotermes* mound in Palapye, Central Botswana (photo: Peter N. Eze).

regulation during cold settings. They move deep down the Earth's surface and hibernate where they become inactive throughout the cold season. They do not have absolute nesting sites and may use any site that can provide warmth like sewerages, logs, and heated structures. *R. flavipes* have therefore been indicated to be capable of surviving prolonged cold temperatures in cold climates out of their range [19].

Trinervitermes trinervoides species are found throughout the Southern African region except Northern Limpopo. Their mounds are dome-shaped and can be as high as 0.9 m high. They are characterized by hard crusts and moist interior, which is channelized in honeycomb-like structure which can go as deep as 46 cm underground. The mounds are closed ventilation systems, meaning they leave no space on the exterior when building their structures. Even though they are grass harvesters, they are not fungus growing; hence, they do not need to regulate the temperatures in their nest for fungus growth. In case of high temperatures, they introduce water in the mound to cool off. The grass fragments collected may be used to insulate their mound-interior as well as providing constant food source.

Two *Macrotermes* species were studied by Kang [12], *M. bellicosus* and *M. subhyalinus*, in South Western Nigeria and a ratio of *M. bellicosus* to *M. subhyalinus* of 8.5 to 1 was reported. *M. bellicosus* are characterized by high mounds of about 195 cm, while *M. subhyalinus* have a large base diameter and are more solid. It is noteworthy that *M. bellicosus* are the most abundant species in the Nigerian Tropical Savannah [20]. Their mound height may be as size as $80 \pm 10 \, \text{m}^3$ and base $1.08 \pm 0.42 \, \text{m}$ [4]. Special termite species that does not digest cellulose, *M. herus*, was studied by Moe et al. [3]. These species grow fungi inside and outside their mounds which they use to digest the plant material, therefore releasing nutrients and minerals, and which are

then taken up by termites as part of their diet. *M. herus* are found to be the most abundant termite species in Mburo National Park ecosystem of South Western Uganda.

Northeastern Brazil has large termite mounds which are densely packed, with diameter of about 9 m. The mounds are prominent landforms in this area. Termite species largely found in this area is the *Syntermes dirus* (Burmeister). Their mounds are circular-shaped with ordered spacing between the mounds with well-controlled temperature in the mounds. Usually when the mounds are about 1.5 m high, they may appear abandoned, while in actual fact they are still there with other termite species and ants coinhabiting the mounds. Other termites may leave the mounds and build a new colony, hence explaining the densely packed mounds in this area [21]. Another species, Odontotermes obesus, largely found in India [13], is commonly known to construct large mounds which can be 2 meters high [11]. The discussed termite species are by no means exhaustive of the total species present but were briefly discussed because of their dominance in tropical soils and landscapes.

3. Properties of Termite Mounds in Relation to the Surrounding Soils

3.1. Physicochemical Properties. Termite mounds like other adjoining soils are made up of sand, silt, and clay particles. However, mounds have been reported to have higher clay contents as compared to the surrounding soils [22, 23]. The clay minerals enhance soil stability in a way that they form strong bonds between particles which hold them together. The activity of migrating finer particles towards the surface also helps to create pores which enhance aggregate stability. While evaluating the properties of modified soils with termite mounds, Asawalam and Johnson [24] reported that modified soil had improved structure, hence more aeration, better water infiltration, and reduced crop root diseases as compared to unmodified adjacent soils.

Chemical nutrients are usually more available in termite mounds than in the surrounding soil. Available nutrients are ammonium (NH₄⁺), nitrate (NO₃⁻) and exchangeable cations [e.g., calcium (Ca²⁺), magnesium (Mg²⁺), potassium (K⁺), and sodium (Na⁺)] [25]. Organic matter is an important biochemical component of the soil [26, 27]. Similar to clay and iron oxides, it plays vital role in soil aggregate formation as a cementing agent. Although termite mounds generally have less organic matter content than the surrounding soils [23, 28], they have higher aluminium, iron, and titanium content as well as their oxides which are effective in the absorption of materials [28]. Soil organic matter (SOM) is considered a cementing agent ensuring the structural stability of mounds, but the poor organic matter content of termite mounds suggests that SOM has a negligible role and that clay can be considered as the major factor responsible for the structural stability of *Macrotermes* mounds [29]. There are several reports where the contributions of soil fauna to soil fertility have been assessed. Some subsistent farmers in Africa use the presence of termite mounds as a signal of soil fertility [30]. Others may go as far as collecting the mound materials and spreading them all over their field to improve the soil properties as well as increasing the fertility of the land.

Local conditions have an effect on structure of the mounds. Relatively, very few studies have been carried on the wetlands because there are more mounds on the upland than in the lowlands [31]. Studies done in a semiarid environment of Southern Africa show that soils on eroded mounds tend to be more acidic with higher concentrations of Mg, Ca, N, and P as compared to soils that occur 0.5 m away from the margins of eroded, inactive, and active mounds [32], a clear evidence that mound building termites consume vegetation, alter soil fertility and drainage, and hence create disturbances. The Luvisols in mounds of Macrotermes in Bandipur Tiger reserve forest, southern India, were found to have low N and C, while Ferralsols had quite the opposite. In summary, termite mounds develop physical and chemical properties that keep them in equilibrium with their immediate environments [11].

3.2. Biological Properties of Termite Mounds. Termites are divided into grass, wood, litter, and soil feeding organisms. They can digest about 27%–90% litter and 4%–13% of biomass above ground level [10]. Macrotermes which are the most abundant in Africa are one example of fungus-growing termites. These termites grow fungi in the litter, therefore releasing the biologically essential N and P which the termites feed on. The litter may also enhance soil organic matter. With their ability to digest lignin from weeds and other municipal wastes, studies show that they can be used to treat solid waste in a process called vermicomposting [33]. Termigradation is the term used to describe the use of termites to dispose solid waste. Other than ability to digest lignin, termites have big appetites, meaning they can consume a lot of waste at a time [34].

Since they have strong division of labour, only workers can digest lignin, and they are also not breeding. Even though they have this special character of digesting lignin, they are difficult to culture and they may not be able to survive if they are separated from their colony. This may be a challenge to vermicomposting. A study by Andrianjaka et al. [35] gives details on the use of mound powder used to control plant parasite and for use in plants. When Cubitermes mound powder was spread over sorghum seedlings, it brought an increment in growth rate. This is due to fertilizing potential of termite mounds due to the minerals (N and P) present. In tropical forests, the biological activity of termites is evident in litter, where some of the litter is buried as they build their structures, therefore adding organic matter. Their guts are alkaline, a feature that allows them to decompose recalcitrant materials.

3.3. Geochemical and Mineralogical Properties. Since termites build their mounds with clays collected from underground, their geochemical composition and mineralogy may or may not be different from their surrounding soils of the termite mounds. A few studies, however, show that there are differences between geochemical composition and clay mineralogy in mounds and surrounding soils. Rare Earth

elements and trace elements including B, Fe, Mn, Ni, Cu, Zn, Se, Mo, and Cd contents are reportedly higher in termite mounds compared to the surrounding topsoils. This enrichment suggests a possible external supply of enriched materials or accumulation of in situ weathering products of the underlying bedrock [36]. Similarly, Ti and Zr enrichment is common in termite mounds and this could be attributed to the decomposition of biotite by Macrotermes with consequent release of Ti and Zr into the mounds, implying the termites' inherent ability to transform soil minerals and enhance chemical weathering [36, 37]. In the sites studied by Mujinya et al. [38], kaolinite was more abundant, with quartz, K-feldspar, and haematite, than the surrounding soils. Termites are involved in the transformation of K-feldspar to kaolinite and synthesis of organometal complexes [8]. Termite mounds used for geophagy by chimpanzees in the Mahale Mountains National Park, Tanzania, showed that the termite mounds had relatively higher aluminium (10.0%), iron (3.0%), and sodium (0.5%) than the surrounding soils and this correlated well with the mineralogy of the clay ($<2 \mu m$) fraction, which is high in metahalloysite, a 1:1 (Si:Al=1:1) clay mineral similar in chemical composition to the clay mineral kaolinite, and smectite (montmorillonite), which is a 2:1 expandable clay mineral. Pharmaceutical Kaopectate™, a substance obtained from the combination of metahalloysite and smectite, is extensively used as antidiarrheal agent [39].

4. Roles of Termites in Ecosystem Functioning

The ecological roles of termites are well known to soil ecologists as a lot of research has been done on this over the years (e.g., [40-43]). This paper will not cover this and readers are referred to a review of the ecosystem services provided by termites by Jouquet et al. [17]. Outstanding roles of termites in the ecosystem include their active involvement in bioturbation and pedogenesis, organic litter degradation and decomposition, water infiltration and runoff, and soil erosion, organic matter and nutrient cycling, soil animal and microbial diversity, and vegetation growth and diversity. Termites further exert more impacts in arid and semiarid environments where they are one of the major soil macro invertebrate decomposers through the building of biological structures including mounds, sheeting, and galleries with varying physicochemical properties. Other ecological and environmental significances of termites not discussed in Jouquet et al. [17] are highlighted below.

5. Termite Mounds and Geochemical Exploration

Termites are actively involved in pedogenesis when they burrow into the subsoil horizons and move soil materials upward (bioturbation) when they build mounds. It is through this mechanism that termites transport ore minerals deposit from subsoils to soil surface. Stewart and Anand [44] reported that some metals indicators are a result of nutrients attained through food sources. In a study carried out at Garden Well, Western Australia, Au deposit in mounds of *Tumulitermes*

tumuli was examined. Au mineralization of 7.4 ppb was detected using the high-resolution particle-induced X-ray emission (PIXE) mapping and scanning electron microscope (SEM) imaging coupled to energy dispersive X-ray (EDX) analyses. Calcite-rich fragments that are linked to Au were also found and Au increased with depth in the mound to 20 ppb. This is so because mineral particles may be diluted by soil fragments from the shallow regions as they are moved upward. A study in North Ethiopia where the soils are mostly lithosols focused on using termite mounds in geochemical exploration. A positive correlation between minerals in termite mounds and in the bedrock was observed and hence considered a simple way of detecting minerals in developing areas like North Ethiopia where thick regolith makes it uneasy to reach the bedrock. There were high correlation for gold, copper, and manganese, respectively, and medium correlation for silver. A negative correlation was recorded for zinc, cobalt, and nickel [45]. It was therefore concluded that termite mounds can be used to in geochemical exploration of certain minerals.

6. Underground Water Treatment and Water Survey

Arsenic is one of the harmful chemicals that can cause long-term effects on human health. The effects may be permanent and deadly like dermal lesion and skin, lung, and bladder cancer. Scientists have looked for ways to remove arsenic in groundwater for safe consumption. Some methods are adsorption, solvent extraction, and iron exchange. Fufa et al. [28] reported that these methods are expensive and may not be applicable in many areas especially the developing countries with low gross domestic product (GDP). The use of termite mounds has been put in place to remove arsenic in groundwater. High Al, Ti, and Fe oxide mineral contents of termite mounds enhace adsorption of contaminants. Termite mounds have been successfully used to remove chromium [46], lead [47], zinc [48], and polycyclic aromatic hydrocarbons (PAH) [49].

Northwestern Ethiopia is characterized by large basaltic dykes as observed by ASTER multispectral imagery [50]. These vertical dykes collect run-off water which is enabled by their fracture density; hence, more vegetation and river and its tributaries are found along the dykes. About 20 mounds were observed along the dykes and none on the lava flows. Therefore it is assumed that scientists were using termite mounds to identify the dykes during their study. The mounds are dome-shaped, have regular spacing, and are 1.5-2 m high, with about 1400-2400 species of termites per mound. It was also observed that the mounds were built not higher than 2 m. According to the observations in [50], termites built their mounds near the dykes as they contain water; therefore there is easier access to moisture. Termites use water to regulate mound temperatures, and bind clays for more stable aggregate structure and fungus cultivation by fungus-growing termites. Some termite species like Ballicositermes natalensis can drill many meters below groundwater in search for moisture. Cooled mounds are not associated with high emissions of carbon dioxide. Water that is accessible by termites is distributed by three channels and stored in two reservoirs which are the main water tables in this area. Water is temporarily stored in the dyke and eventually filled up from the water table. It is for this reason that termites build mounds near dykes for continuous availability of moisture even in dry seasons.

7. Thermoregulation, Gas Exchange, and Global Climate Change

The shape of a termite mound is determined by the habitat and the function of the mound. The amount of heat lost or produced will also be determined by the shape of the mound. In the savanna biome, where there is aridity of the climate, the mounds are usually dome-shaped with thin walls to allow air to escape the nest. Again in the forests where temperatures are cool the mounds are dome-shaped with thick walls to reduce heat loss through the mound. The differences in temperature affect termites' population and density. Termites develop a long-term internal mound temperature control as it is easier to control over external temperature [51]. Warm temperatures are associated with higher mounds and high reproduction. Macrotermes falciger in the Democratic Republic of Congo lives in a woodland environment where it is warm and humid. In response to changes in climate, they build their mounds in a closed dome structure with thick walls and low surface area. This arrangement helps to keep their structures warm and humid with efficient gaseous exchange with their environment. They prefer temperatures of ±30°C [18]. When there are low gaseous exchange and low temperatures, metabolic rate decreases, hence low production and vice versa. The species Macrotermes bellicosus keeps its internal temperature at about 30°C throughout the year for optimum fungus growth. A comprehensive discussion on different termite species and how they regulate temperature is described by Field and Duncan [51]. Larger termite mounds have high heat retention capacity as compared to smaller mounds because of the area and the level of metabolic activity taking place. They are more insulated and greater metabolic activity means more heat production.

Studies have shown that rainfall and temperatures are the major active factors of climate that influence the distribution of termite mounds over large areas [52]. High concentration of greenhouse gases in the atmosphere contributes to the increase in global temperature. Termites release methane gas which originates from the fermentation of organic matter [53] during the construction of mounds. About 1/3 of methane is released from natural sources including oceans and termite mounds while two-thirds are from man-made sources like burning of biomass. Annually, about 580 Tg of methane is produced by termites [9]. Savanna biome covers 30% of world vegetation and approximately 61% of macro invertebrates with termites included live in this biome. The two feeding patterns of termites, the soil feeding and that

which feeds on vegetative parts of plants (wood, leaves, grass, etc.), ensure that termites digest their food through anaerobic fermentation where methane is produced. Soil feeders produce more methane [54]. Termites contribute between 0.2 and 2% CO₂ and about 30% of methane in the savanna biome. Asia alone is covered with 12% of world's savannah [55]. Different researchers have reported different values of methane emission due to changes in factors or parameters used to measure emissions, for example, fecal content and lack of measured emissions in the field [9]. A study from Bréas et al. [54] shows that global emission of methane by termites is 0.3% to 1.5% of global methane. Jamali et al. [9] reported that between 5 and 19% of global methane is produced by termites. Both the mentioned results are said to be lower than those estimated by IPCC [56]. These statistics show the proportion of greenhouse gases (especially methane) contributed by termites in the atmosphere, even though the statistics are questioned and believed to be overestimate. Warm temperature results in more methane production in termites, so increase in global temperature will lead to more production by termites. Disturbed termites produce more CO₂ than they would normally do [57]. On the other hand, termites may be seen as pests in the field. People in some parts of the world where they use low cost technology for weather forecasting use termites as climate indicators. The presence and abundance of subterranean termites may indicate that there will be severe drought [57]. Termite mounds therefore are strongly linked with thermoregulation of human environments, gas exchange, and global climate change phenomena.

8. The Study of Termite Mounds at Nanoscale, a Worthy Research Direction?

From structural point of view, the soil material has multiple scales with features and properties peculiar to each scale level. It is against this backdrop that Zinck [58] used a hierarchical model (Table 1) to introduce some basic soil notion. Soil material is considered in its elementary form of molecules and combinations of molecules into particles at nanoscale.

Basic reactions of the soil material including chemical (solution, hydrolysis, carbonation, hydration, and redox), mechanical (types of packing and types of fabrics (e.g., flocculated, deflocculated, dispersed, and aggregated fabrics)), and physicochemical which are reactions are based on the colloidal properties of clay and humus [59]. These reactions control important Earth surface process including chemical weathering, pedogenesis, contaminant transport, nutrient availability, mass movement, and other soil erosion phenomena in soils and landscapes.

At the turn of the twenty-first century, new advances in the instrumentation paved way for scientists to study objects at the scale of 1 to ~100 billionth of a meter, known as the nanoscale [60]. Most of the natural components of termite mounds are nanoparticles including humic substances, phyllosilicate clays, Fe(hdr)oxides, and ferrihydrite. Nanoparticles play a vital role in nutrient bioavailability, pollutant

Level	Unit	Concept	Soil feature
Nano	nm-μm	Particle	Basic soil reactions
Micro	μm-mm	Aggregate	Micromorphological structure
Meso	mm-cm-dm	Horizon	Differentiation of the soil material
Macro	m	Pedon	Soil volume for description and sampling
Mega	m-km	Polypedon	Soil classification and mapping (geo)pedologic landscape

TABLE 1: Hierarchic levels of the soil system [58].

transport, and structural stability of termite mounds [61] and mediate biogeochemical processes with global relevance, such as turnover of the important greenhouse gas methane (CH₄). However, the complex internal and external morphologies of termite mounds impede an accurate quantitative description of gas emission. To understand the properties and functioning of the various sizes of termite mounds existing across the globe, a realistic picture of the entire solid and pore (void) structures is fundamental. However, due to spatial and temporal variations in the nature of termite mounds built by different species in different soils, most analytical techniques have respective shortcomings and provide only a limited understanding of the termite mound system. To gain a more comprehensive understanding of the nanoscale structure of termite mounds, soil scientists, over the years, have resorted to approaches ranging from the examination of thin sections of termite mound hand samples under petrographic microscopes (e.g., [62]). In micromorphology approach, thin sections are prepared from the undisturbed samples, after impregnation with stained polyester blue resin [63]) after which micromorphological features of the termite mounds are observed under transmitted plain light, whereas the isoand anisotropism of the materials, and the birefringence fabrics of the fine material, are observed under polarized light. Optical microscopy can provide micropedological evidence for the biological formation of microstructure [64, 65], granulometry, and porosity [8]. With advances in science and technology, more robust techniques for examination of the nanoscale structures of termite mounds have evolved. These approaches include the following.

8.1. Midinfrared Spectroscopy (MIRS). This is a very fast, high-throughput method for characterising the chemical composition of materials including termite mounds. To apply this method, a sample of termite mound is illuminated and the diffuse reflected light (electromagnetic radiation) is measured in narrow wavebands over the range of $4,000-400 \,\mathrm{cm}^{-1}$ (2,500-25,000 nm) and the resulting spectral signature summarises how much energy was absorbed at each wavelength. The recorded spectral signatures respond to soil organomineral compositions of the termite mound. Specific absorption features of the MIRS can also provide details about the molecular structure of the termite mound, for example, organic matter quality. In their work to analyse the biochemical fingerprints of termite colonies (soldiers and minor and major workers) and to differentiate termite colonies in terms of geographical distances between colonies and/or their ages, the authors in [66] successfully applied mid-infrared spectroscopy (2500-25000 nm). The technique

further highlights the close relationship between the physiological states of termite colonies and their environment. To establish correlations between soil and termite mound parameters and MIRS variables, statistical procedures including principal component analysis (PCA) and partial least squares regressions (PLSR) are often used [66, 67]. MIR could provide an added advantage of providing a rapid, lowcost, and nondestructive method of analysing soils without the use of chemicals.

8.2. Photogrammetry and Cross-Sectional Image Analysis. To understand the complex internal and external morphology of termite mound in order to properly quantify methane, Naeur et al. [68] presented a novel method that combines photogrammetry with cross-sectional image analysis. Termite mound parameters including surface area, basal area, and epigeal volume were measured by reconstructing 3D models from digital photographs and compared against a water-displacement method and the conventional approach of approximating termite mounds by simple geometric shapes. Macro- and microporosity (θM and $\theta\mu$) were introduced to internal structure of termite mounds, the volume fractions of macroscopic chambers, and microscopic pores in the wall material, respectively. As with soil samples, macro- and microporosity represent the total porosity of termite mound, which denotes the portion of termite mound occupied by water and air. Macroporosity and complete pore fractions were successfully estimated using image analysis of single termite mound cross sections and compared against full X-ray computer tomography (CT) scans and assess species-specific differences in internal structure of the termite mound. This novel technique improved the estimation of CH₄ emission into the environment but does call for more trials with termite mound from other termite species as comprehensive CT scanning revealed that investigated termite mounds have species-specific ranges of θM and $\theta \mu$ but similar total porosity. The new image-based methods allow rapid and accurate quantitative characterisation of termite mounds to answer ecological, physiological, and biogeochemical questions. The success of the photogrammetry method proves that it can be applied to limit large errors from inconsistent shape approximations when measuring greenhouse gas emissions from termite mounds [68].

8.3. Transmission Electron Microscopy (TEM) and Pyrolysis Field Ionization Mass Spectrometry (Py-FIMS). In TEM, the applied high-energy beams of electrons are reflected by the thin gold or platinum coating of termite mound, and the

interactions between the electrons and the atoms can be used to observe features at nanoscale. Pedofeatures such as voids and grain boundaries within the soil matrix can be observed in TEM. A combination of TEM and Py-FIMS has been used to better understand the role of SOM and inorganic colloids in the stabilization of carbon and nitrogen at the nm scale [69]. Observations made by TEM indicated that SOM in nanosize fractions occurred mainly in organomineral complexes but also in solitary structures of humic substances that formed a carbonaceous network of single strands linking clusters of humic materials and minerals, ranging from a few nm to μ m in size [69].

8.4. Scanning Transmission X-Ray Microscopy (STXM) Using Synchrotron Radiation in Conjunction with Near-Edge X-Ray Absorption Fine Structure (NEXAFS) Spectroscopy. To tackle the methodological limitations to which existing soil aggregation models explain carbon (C) stabilization in soils and poor quantification of small-scale heterogeneity of organic carbon, Lehmann et al. [70] developed a technique applying a combination of STXM and NEXAFS to investigate (50 nm resolution) C forms in black C particles and performed a comparison to synchrotron-based FTIR spectroscopy. For the first time, the nanoscale biogeocomplexity of unaltered soil microaggregate was resolved using STXM and C 1s-NEXAFS and we believe this can also be applicable to termite mounds. Aggregation and stability of termite mound determine its ability to resist deformation under applied external pressure. Physical infrastructure of microaggregates also plays a fundamental role in determining the chemistry of the occluded C and intimate associations between particulate C, chemically stabilized C, and the soil mineral matrix. Apparently, NEXAFS spectroscopy has great potential to improve our understanding of black C properties which has important implications for biogeochemical cycles including mineralization of black C in soils and sediments and adsorption of C, nutrients, and pollutants as well as transport in the pedosphere, geosphere, hydrosphere, and atmosphere [70, 71].

8.5. High-Resolution Magic Angle Spinning Nuclear Magnetic Resonance (HR-MAS NMR). Structures at the solid-aqueous interface of a whole soil can be examined by the use of high-resolution magic angle spinning nuclear magnetic resonance as demonstrated by Simpson et al. [72]. This methodology allows the application of solution-state NMR experiments to samples that are not completely soluble and contain solids, for example, soils. The identification of fatty acids, aliphatic esters, and ethers/alcohols as outstanding species at the solid-aqueous interface of soil with signals from sugars and amino acids, for the first time, became possible following the combination of one- and two-dimensional HR-MAS NMR. Therefore, HR-MAS is a promising technique that can be widely applicable to a range of complex environmental samples without the need for extraction, pretreatment, or purification [72].

Given the ubiquity of termite mounds in the tropics and semiarid environments, future research on their nanoscale particles should seek to understand the behaviour of water and gases in nanopores, the mechanisms of nanotoxicity, how termite mounds can be used to clean up potentially toxic chemicals and pollutants out of the food web, and how the complex interaction of soil organic matter, mono- and multivalent ions, and microorganisms affects aggregation in particular and pedogenesis at large.

9. Conclusion

Termite mounds are common landscape feature in the tropical climates of the world. They are important biological structure built by termites to protect them from harsh environmental conditions. The size and structure of termite mounds vary across climate gradients and are also dependent on the species of termite involved. Termite mounds vary in physical, geochemical, biological, and mineralogical properties from the adjoining soils. They play outstanding roles in groundwater treatment, geohydrology, geochemical exploration, thermoregulation, gas exchange, and global climate change. Termite mounds are replete with nanoparticles from organomineral complexes, but there are still methodological limitations on how much examination of termite mound can be done by soil ecologists. In this review, we present the documented successful techniques that include mid-infrared spectroscopy (MIRS), photogrammetry and cross-sectional image analysis, a combination of transmission electron microscopy (TEM) and pyrolysis field ionization mass spectrometry (Py-FIMS), scanning transmission X-ray microscopy (STXM) using synchrotron radiation in conjunction with near-edge X-ray absorption fine structure (NEXAFS) spectroscopy, and high-resolution magic angle spinning nuclear magnetic resonance (HR-MAS NMR). There is a need to continually develop and integrate nanotechnology with the routine classical soil analysis methods to improve our understanding of the functional mechanisms of organomineral complexes of nanostructure of termite mounds that are responsible for specific properties. This will ensure proper use and management of termite mounds in achieving agricultural, environmental, and engineering feats.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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