

Testing Infrared Technique to Discriminate Indigenous Termite

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Abstract

As the most abundant and ubiquitous soil macrofauna, termite fauna is identified using indigenous and scientific criteria. The study objective was to test the infrared technique to discriminate termite species identified by vernacular knowledge. Fresh termites were collected through indigenous harvest process and hereafter termites were identified using morphometric data and their signatures in infrared based carbon and nitrogen content in termite dry biomass. A total of 16 termite species grouped in 7 genera, 3 subfamilies and in 4 feeder groups were morphometrically identified. The mainly genera were *Macrotermes*, *Nitiditermes*, *Odontotermes* and *Trinervitermes* whereas *Macrotermitinae* with 4 genera and 8 species were the most frequent and abundant subfamily. Next, *Cubitermitinae* and *Nasutitermitinae* were represented by 6 species with an updated genus of *Nitiditermes* and 2 species. *M. bellicosus*, *M. subhyalinus*, *O. vulgaris* and *T. trinervius* were discriminated by carbon and nitrogen rate of their dry biomass powder. In conclusion, infrared technique could be used as tool for rapid discriminating vernacular termite species and therefore should be taken into account in studying termite biodiversity management and conservation.

Keywords: Biodiversity, Burkina Faso, Chemical Component, Savannah, Species Diversity, Soil Macrofauna

1. Introduction

Termite fauna is well recognized for being the most abundant and expanded soil macrofauna in savannah ecosystems and farmlands in tropical and subtropical areas [1,2]. Termite colony can host up to millions of individuals m⁻², being 95% of the total biomass of soil macrofauna in some environmental conditions referring [3,4]. Meanwhile in literature, data available on termite activities and their functional impacts are contradictory and controversial and at times, conflicting. Termites sometimes stand out by their negative impacts through the infestation, damage or destruction of the different part of plant species and annual crops at all the stages of their growth [5,2,6-8]. At other times, termite fauna stands out as positive ecological actor exerting direct and indirect valuable effects over natural ecosystems and farmlands and being ecosystem goods for local residents [9-12].

Previous studies have reported that silviculture tools and land use type and land conversion practices can deeply alter termite species composition, nest-building and foraging behaviour, and so the colony development through habitat and food resource modification [13,9,14,15]. During their studies, different techniques have been used for sampling termite species as quadrat plots under treatment, soil digging, dead or fresh wood collecting or randomised point scales which efficiently have allowed assessing the species richness and feeding groups of termites encountered. However, they have not combined scientific techniques and termite identification criteria of indigenous knowledge in species composition appraisal. The knowledge of termite species and their behaviour remain a priority for sustaining conservation and efficient integrated management of termite fauna. This study endeavour to analyse if indigenous knowledge can play a key role in identifying the different groups of termite assemblage for the general assessment

of their assemblage and behaviour. Specifically, to identify termite composition based on i) vernacular name and morphometric data and, ii) to discriminate termite species using infrared technique for termite signature. We assumed that the infrared and organic C and N content could be used to identify indigenous termite biodiversity.

2. Methods

2.1. Study Sites

Termites were sampled in a state forest and in an open agricultural land where rice paddy is cultivated in June 2011, May 2012 and August 2016. Vegetation is a tree and bush savannah dominated by species as *Andropogon gayanus* Kunth, *A. pseudapricus* Stapf, *Loudetia togoensis* (Pilg.) C. E. Hubb. and *Pennisetum pedicellatum* Trin for grass layer, and by Caesalpiniaceae, Combretaceae and Mimosaceae families for woody stratum. Rainfall was highly various and occurred generally from April to October with an average of 919 mm per annum.

2.2. Experimental Design

To identify their genus, fresh termites were harvested in 2011, 2012, and in 2016, involving the mid of rainy season, and the period of termite high activities of mound repair and food harvest for storage. Experimental plans were set up using empirical methods of new and intact baited terracotta canaries as artificial mounds (Fig. 2. a and b).

As baits, the dry dung of cattle, the dry straw of *Z. mays*, *L. togoensis* and *Rottboellia hirsuta* Vahl were used to fill these canaries up while control canary was set using all the substrata at once. All the substrata were moistened and maintained using 2 fresh branches of *Diospyros mespiliformis* Hochst. Ex A.D.C. cross inserted in each container. Canaries were hereafter returned and deposited. The content of each canary was collected after 2 days, and transported to laboratory and termite individuals were stored in a 70% ethanol solution in the flask of 50ml and kept fresh in refrigerator for being later identified.



Figure 1: Set of Experimental Canaries and Substrat for Termites Harvesting in Fields

2.3. Termite Identification

Sampled termites were first sorted out based on vernacular name, and identified by genus in the laboratory of Plant Biology and Physiology of University Nazi BONI. All the termites genus were later updated in the laboratory of Evolutionary Biology and Ecology, ULB, Belgium, using the morphometrical parameters according to determination keys in use [16-22]. Soldier individuals even worker members were mainly used to discriminate termite genus and species. Our samples were checked using specimen and species name and later updated crossing [23,24]

Infrared techniques of were at last performed to discriminate termite species. About 100 fresh individuals 50 soldiers and 50 workers of *Macrotermes bellicosus*, *M. subhyalinu*, *Trinervitermes trinervius* and *Odontotermes vulgaris* were sampled and oven-dried at 105°C during 48 hours considering their body mass and holding their digestive system of which the content can significantly be informative for their organic carbon (C) and nitrogen (N) content [25]. The dry biomass was manually ground to a fine powder <1 mm of which five replicated samples of 1 g were scanned with a spectrometer (Matrix-F, Bruker) in the 1100e

2500 nm spectral range and three replicate samples of 3g of termite powder were sealed and incinerated to evaluate the rate of organic C and N content using a CHN analyser as Elementar Vario EL III usually used for soil to complete infrared results. The infrared and incineration techniques with a CHN analyser were performed at the laboratory of BioPhys, iEES- IRD, Bondy, France.

Principal component analysis (PCA) was performed as methods to graphically discriminate termite species and regression analysis for global C and N content. The one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls' tests were applied for the multiple comparison of organic C and N, and C/N ratio between termite species at the 5% level of significance.

3. Results

3.1. Termite Species Composition

As total of global data, four types of termites in different local names based on termite body size, head and stomach colour, and mound external shape were identified (Table 1). Thereby, black termites, builders of mushroom-shaped mound referred to *Cubitermes* spp while small white termites with a sharpened mouth

(labrum) builders of sand lot mounds referred to *Trinervitermes* spp. Termite individuals with a big red head and great black pincers (mandibles) erecting huge epigeal mounds with closed turrets were recognized as *Macrotermes* spp and those with red head and building huge epigeal mounds with opened chimney were identified as *Odontotermes* spp. The table 1 summarized

and presented termite species identified using simultaneously vernacular and laboratory criteria. Based on morphological traits and morphometric data in photonic microscopy, a total of 7 genera of Termitidae family were identified including *Macrotermes* spp, *Nitiditermes* spp, *Odontotermes* spp and *Trinervitermes* spp according to termite soldier individuals from pooled data [24].

Termite scientific name	Local name	Feeder group
Cubitermitinae		
<i>Nitiditermes falcifer</i>	Black termites	Humivorous
<i>Nitiditermes umbratus</i>		
<i>Nitiditermes</i> sp3		
<i>Nitiditermes</i> sp4		
<i>Nitiditermes oculatus</i> or <i>niokoloensis</i>		
<i>Nitiditermes proximatus</i>		
Macrotermitinae		
<i>Ancistrotermes cavithorax</i>	Small white termites	Fungus-grower
<i>Macrotermes subhyalinus</i>	Big red head and great black hook/teeth	Litter-feeders, fungus-grower
<i>Macrotermes bellicosus</i>		
<i>Microtermes subhyalinus</i>	Small and white termites	
<i>Odontotermes pauperans</i>	Small and red head termites	
<i>Odontotermes vulgaris</i>		
<i>Odontotermes</i> sp3 <i>culturatum</i> or <i>monodon</i>		
<i>Ophiotermes</i> sp	Small white termites	Fungus-grower
Nasutitermitinae		
<i>Trinervitermes oconomus</i>	Small white termites	Grass-feeders
<i>Trinervitermes trinervius</i>		

Table 1: Complete List of Termite Species Recorded in the Study Site During Study Period

Among these genera, 16 species were noted and gathered in 3 subfamilies and 4 feeding groups. Macrotermitinae subfamily was the most represented with 4 genera (57.14%) and 8 (50%) species. One (14.29%) genus in each of the subfamilies Cubitermitinae with 6 species (37.5%) and Nasutitermitinae with 2 species (12.5%). Based on mound building profile, eleven species as *Macrotermes* spp, *Nitiditermes* spp, *Trinervitermes* spp and *Odontotermes* spp were identified as mound builder termites while the other species were considered as underground termites. Genera as *Ancistrotermes* and *Microtermes* represented each by 1 species as *A. cavithorax* (Sjöstedt), *Microtermes subhyalinus* (Silvestri) and “*Ophiotermes*” were collected from the mounds of *Nitiditermes falcifer* (ex *Cubitermes falciger*). *Ancistrotermes* sp, *Microtermes subhyalinus* and *Ophiotermes* sp were called white and black (for *Ophiotermes*) termites in addition to their size. All the described features of this last termite soldier referred to *Ophiotermes* sp (specimen is holding by the first author) having both a prominent forehead and a bifurcated labrum similar to that of *Nitiditermes*

spp soldier.

At baiting level, 7 species of Macrotermitinae except *A. cavivorax* were captured by the combined materials used for the control terracotta canary. *Macrotermes* sp and *Odontotermes* sp were only found in canaries filled with both of the dry dung and straw or alone whereas *Trinervitermes* termites were solely trapped with grass biomass.

The individuals of *Trinervitermes* sp and *Ophiotermes* sp were only obtained with the canaries containing the dry straw of *Z. mays*, dry biomass of *L. togoensis* or *R. hirsuta* that had previously been moistened while those of *Macrotermes* sp and *Odontotermes* sp were separately obtained each with dry cattle dung moistened as a clue of interspecific competition. *Nitiditermes* sp. No member of *Nitiditermes* spp and *A. cavivorax* was encountered outside their mounds. They had only been taken from their mounds.

Termite scientific name	Local name	Feeder group
Cubitermitinae		
<i>Nitiditermes falcifer</i>	Black termites	Humivorous
<i>Nitiditermes umbratus</i>		
<i>Nitiditermes</i> sp3		
<i>Nitiditermes</i> sp4		
<i>Nitiditermes oculatus</i> or <i>niokoloensis</i>		
<i>Nitiditermes proximatus</i>		
Macrotermitinae		
<i>Ancistrotermes cavithorax</i>	Small white termites	Fungus-grower
<i>Macrotermes subhyalinus</i>	Big red head and great black hook/teeth	Litter-feeders, fungus-grower
<i>Macrotermes bellicosus</i>		
<i>Microtermes subhyalinus</i>	Small and white termites	
<i>Odontotermes pauperans</i>	Small and red head termites	
<i>Odontotermes vulgaris</i>		
<i>Odontotermes</i> sp3 <i>culturatum</i> or <i>monodon</i>		
<i>Ophiotermes</i> sp	Small white termites	Fungus-grower
Nasutitermitinae		
<i>Trinervitermes oeconomus</i>	Small white termites	Grass-feeders
<i>Trinervitermes trinervius</i>		

Table 2: Complete List of Termite Species Recorded in the Study Site during Study Period

3.2. Infrared Spectra and Termite Species

The infrared spectrum (Fig. 2) showed and summarized the difference between the identified termite species according to their signatures. The absorbance values varied as the function of the wavelengths (nm) of the electromagnetic radiation expressing the organic C and N contents in the dry biomass of termite samples as presented and summarized in Fig. 2. The coefficient of correlation indicated that $R^2 = 74\%$ of the variation were explained by the higher rate of N (Fig. 3a) and $R^2 = 45\%$ (<50%) by C content (Fig. 3b). Therefore, $R^2 = 71\%$ of variation resulted from C/N ratio values (Fig. 3c). These differences in the absorbance spectra were statistically certified using ANOVA analysis at $P < 0.5$. *Macrotermes*

spp, *Odontotermes* spp, *Trinervitermes* spp were chemically different the one of the other as certified by the amount of C ($F = 5.25$; $P = 0.012$), N ($F = 10.94$, $P = 0.001$) and C/N ratio ($F = 6.58$; $P = 0.005$) that were significantly different between termite species according to table 2. The highest rate of C and N were observed for *T. trinervius* and *M. subhyalinus* whereas the value of C/N ratio was significantly increased for *M. bellicosus* and *O. vulgaris* dry biomass due to the low amount of N in comparison with *M. subhyalinus* and *T. trinervius* showing almost the similar value of C/N ratio (or 4%). At last, the value of C/N ratio significantly increased for *M. bellicosus* and *O. vulgaris* due to the low values of N in their body.

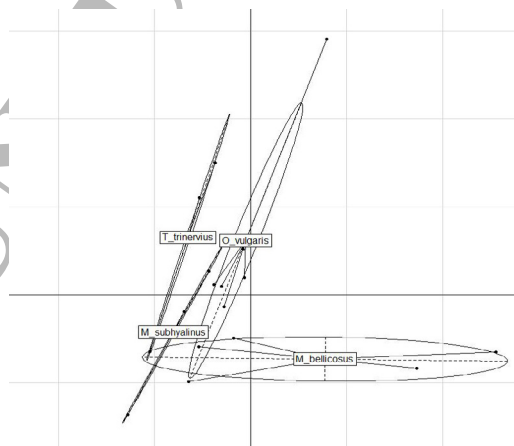


Figure 2: Termite Species Composition (PCA) According to the near Infrared Absorbance Spectrum

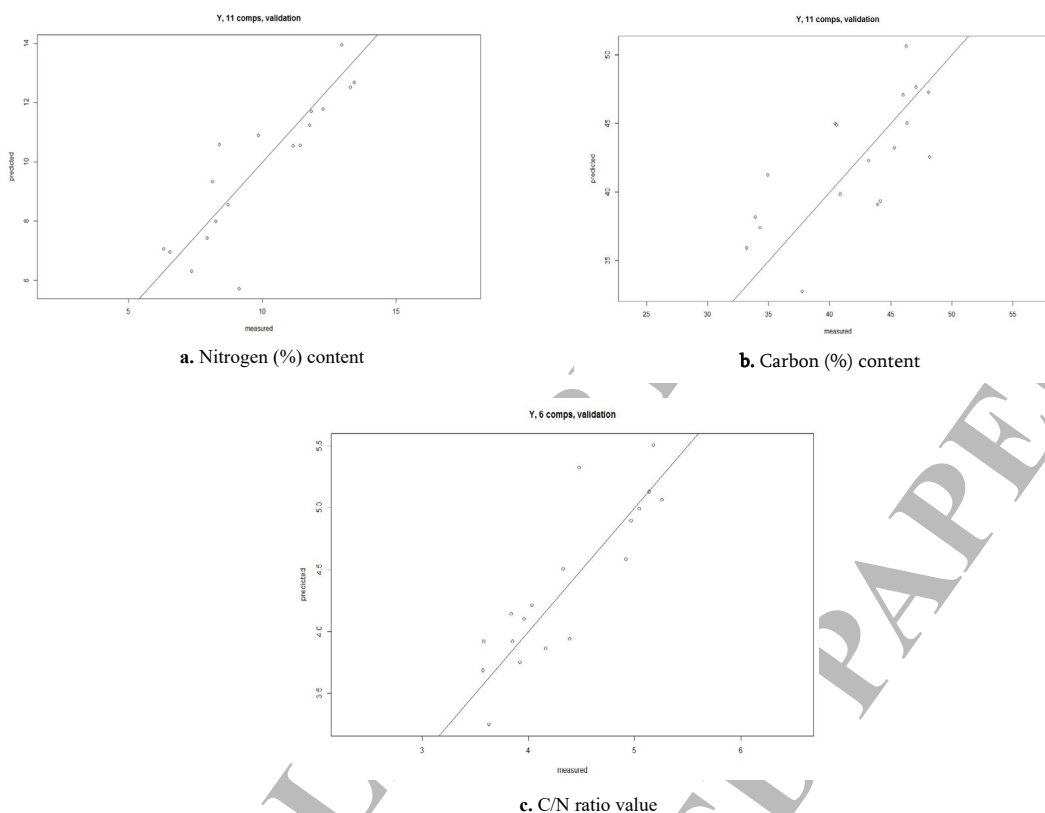


Figure 3: Regression Lines of Infrared Absorbance According to Nitrogen and Carbon content in Dry Termite Biomass

Termite species	Caste	C%	N%	C/N ratio
<i>Macrotermes bellicosus</i>	Worker	34.95±2.46	6.74±0.55	5.19±0.06
	Soldier	41.70±5.95	10.32±1.67	4.05±0.10
	Total	38.33±5.50a	8.53±2.25a	4.62±0.63b
<i>Macrotermes subhyalinus</i>	Soldier	46.24±0.88a	11.96±0.27a	3.87±0.04b
<i>Odontotermes vulgaris</i>	Worker	39.45±4.61	8.97±0.97	4.40±0.08
	Soldier	41.64±1.98	8.37±0.30	4.98±0.06
	Total	40.55±3.39ab	8.67±0.72a	4.69±0.32b
<i>Trinervitermes trinervius</i>	Worker + soldier	47.52±1.08a	13.23±0.24a	3.59±0.03b

Table 3: Organic C and N Content and C/N Ratio (mean±standard deviation %) in the Dry Biomass of the Representative Termite Species

Means with the same letter(s) across the same variable and the same column are not significantly different ($P>0.05$) according to the test of Student-Newman-Keuls.

4. Discussion

Our study highlighted the key contribution of indigenous knowledge to obtain different termite species. The results reported four termite genera as *Macrotermes*, *Nitiditermes* (formerly *Cubitermes*), *Trinervitermes* and *Odontotermes* by the favour of both the colour and size of head, the stomach and body of soldier cast and the shape of mound if any. Like that, the black termites and epigeal mushroom shaped mounds were recognized

as *Nitiditermes* spp of which the members show a black stomach and small white termites as *T. trinervius* building epigeal sandy mounds, *Ancistrotermes* and *Microtermes* with a white stomach. Black stomach termite likely results from the consumption of soil or humus as plant debris and other organic material partially decomposed while the white stomach stores grass or litter [1]. The soldier of *Ophiotermes* sp identified in mound with similar appraisal even though it is locally unknown.

As for genera *Macrotermes* and *Odontotermes*, the soldiers were further illustrated by the colour of their heads and the structure of their "sharp teeth" as labrum. The basic identification keys

of genus, family and subfamily described and, currently used, illustrated vernacular criteria on termite species [3,4,23,24]. Considering specific external features, a total of 16 species were morphologically and morphometrically identified. Grass feeder termites were expected to highly increase in cultivated area due to the availability of dry straw of rice, which was the case in Bama. Surprisingly, it is rather a high species richness of litter-feeder species that has been harvested with dry dung, followed by a total absence of their mounds in the vicinity.

Besides, the results pointed out that *M. bellicosus*, *M. subhyalinus*, *O. vulgaris* and *T. trinervius* are significantly distinct species through the chemical constitution of their body regarding the values of correlation coefficient $R^2 = 74\%$ and $R^2 = 71\%$ of N and C/N respectively. Furthermore, the C and N budget strongly increased from *T. trinervius*, and *M. subhyalinus* soldiers followed by *M. bellicosus* and *O. vulgaris* attributable to the nature of food as grass or dry dung that termites have consumed and stored in their gut (Donovan et al., 2001) before being analyzed. Organic C and N data revealed that *M. subhyalinus*, *M. bellicosus*, *O. vulgaris*, *T. trinervius* are four distinct genera. Our findings are in accordance with those of [26,15,24]. After crossing morphometric and molecular phylogeny data to redescribe termite species have analyzed soldier caste specimen through 5 genera and 3 subfamilies and reported that subfamilies as Amitermitinae, Macrotermitinae, Nasutitermitinae and Rhinotermitinae are evolutionarily distinct, monophyletic genera [26]. Furthermore, figured out that genus *Nitiditermes* is really the genus occurring in West Africa under the refutable genus *Cubitermes* whereas have successfully identified 19 species [24,15]. As outcomes, this study provided new insight in identifying termite species although a lot of researches on termite are limited to genus [27-32].

Infrared technique may play a fundamental role as tool in discriminating indigenous termite species. Hereafter, chemical features may be used to confirm indigenous criteria in handling taxonomic questions in the study of termite fauna for sustaining biodiversity conservation and management.

5. Conclusion

This study showed the contribution of infrared technique in identifying termite species detected and first described by indigenous knowledge. Four main genera as *Macrotermes*, *Nitiditermes*, *Odontotermes* and *Trinervitermes* were locally recorded and 16 species, *Macrotermes* spp, *Nitiditermes* spp, *Odontotermes* spp and *Trinervitermes* spp were scientifically recorded with the help of indigenous methods of termite searching. For the need to trap a wanted genus, *Odontotermes* spp and *Macrotermes* spp were attracted by dry dung and/or straw and *Trinervitermes* spp by dry grass biomass.

As expected, infrared technique could be used as tool for rapid discriminating vernacular termite species across termite assemblages in the sense that the first makes possible to deeply describe termite species and the second to obtain basic data on termite assemblage. These results support the assumption of

which infrared technique should be considered as tool for rapid identification of vernacular termite species when studying termite biodiversity and assemblage throughout the ecosystems and thereby, should be taken into account in the global strategies of termite fauna management and conservation.

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Conflict of interest

The authors declare that there is not any financial conflict of interest or personal relationships that could have influenced the results included in this paper.

Data Availability Statement

All data supporting the results of this study are available from the corresponding author on decent demand.

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