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Shifts in soil faunal community structure in shaded cacao agroforests and consequences for ecosystem function in Central Africa

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Abstract: We assessed the effects of fungicide application on soil fauna in shaded *Theobroma cacao* agro-forests in Cameroon and compared this with forest. Fewer termite species were found in cacao (19) than in forest (29). Species absent in cacao included specialist soil-feeders (*Apilitermes*, *Crenetermes*, *Cubitermes*) and specialist wood-feeders (*Microcerotermes, Nasutitermes*). Conversely, more generalist soil-wood-interface-feeders (*Termes, Amalotermes)* were more frequent in cacao. Mean earthworm densities were 80 m^{-2} in the forest and 81 m^{-2} in the unsprayed cacao, significantly higher than in either low or high sprayed cacao treatments (52 m^{-2} and 57 m^{-2} respectively). The dominant forest epigeic, *Eminoscolex lamani* was absent in cacao. *Rosadrilus camerunensis,* the sole anecic and dominant cast producer had reduced population in cacao and was absent in the high fungicide treatment. The role of this species in ecosystem functioning is highlighted and methods to maintain population, including improving litter quality and reducing soil contamination discussed.

Resumen: Evaluamos los efectos de la aplicación de fungicida sobre la fauna del suelo en agrosistemas forestales de *Theobroma cacao* en Camerún y los comparamos con lo que sucede en el bosque. Hubo menos especies de termitas en el cacao (19) que en el bosque (29). Las especies ausentes en el cacao incluyeron algunas que están especializadas en comer suelo (*Apilitermes*, *Crenetermes*, *Cubitermes*) y especializadas en alimentarse de madera (*Microcerotermes, Nasutitermes*). Por el contrario, las que se alimentan de la interfase suelo-madera, que son más generalistas (*Termes, Amalotermes*), fueron más frecuentes en el cacao. Las densidades promedio de lombrices de tierra fueron 80 m^{-2} en el bosque y 81 m^{-2} en el cacao no rociado, significativamente mayores que los tratamientos de cacao rociados, tanto débil como fuertemente (52 m^{-2} y 57 m^{-2}, respectivamente). La especie epigea dominante del bosque, *Eminoscolex lamani*, estuvo ausente en el cacao. *Rosadrilus camerunensis,* la única especie anécica y productora dominante de deyecciones, tuvo poblaciones reducidas en el cacao y estuvo ausente del tratamiento fuerte de fungicida. Se destaca el papel de esta especie en el funcionamiento ecosistémico y se discuten los métodos para mantener a sus poblaciones, incluyendo el mejoramiento de la calidad del

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mantillo y la reducción de la contaminación del suelo.

Resumo: Avaliaram-se os efeitos da aplicação de fungicidas na fauna do solo em agro-florestas sombreadas de *Theobroma cacao* nos Camarões em comparação com a floresta. Encontrou-se menor número de espécies de térmitas no cacaual (19) do que na floresta (29). As espécies ausentes no cacaual incluem especialistas que se alimentam de solo (*Apilitermes*, *Creneterms*, *Cubitermes*) e espécies especialistas lenhívoras (*Microcertermes*, *Nasutitermes*). Pelo contrário espécies mais generalistas que se alimentam na interface solo-madeira (*Termes*, *Amalotermes*) eram mais frequentes nos cacauais. As densidades médias de minhocas eram de 80 m^{-2} na floresta e 81 m^{-2} nos cacauais não tratados, valores estes significativamente mais altos que com os verificados quer sob baixos ou fortes tratamentos (52 m^{-2} e 57 m^{-2}, respectivamente). A espécie epigéica dominante na floresta, a *Eminoscolex lamani*, encontrava-se ausente no cacaual. A *Rosadrilus camerunensis*, a única espécie anécica moldadora dominante, apresentava populações reduzidas no cacaual e encontrava-se ausente nos que eram fortemente tratados com fungicidas. O papel desta espécie no funcionamento do ecossistema é salientado e os métodos para manutenção das populações, incluindo a melhoria da qualidade da folhada, e para a redução da contaminação do solo é discutida.

Key words: Cameroon, Central Africa, earthworms, humid tropics, *Rosadrilus camerunensis,* termites, *Theobroma cacao,* seedbanks.

Introduction

Chapin III *et al.* (2000) estimated the relative effects of climate, land use change, invasive species, and N/C cycle changes on biodiversity across global biomes and concluded that the most significant factor in both tropical humid forest and grassland biomes was land use change. Grieg-Gran (2006) reported that deforestation in Cameroon is driven by smallholder shifting cultivation. Certainly, smallholders clear land for agriculture, however, net clearance requires subtracting the amount of land that is returning to forest fallow and this is often overlooked. Robiglio & Sinclair (2007) compared the aerial photos from the 1950s, and satellite imageries of 1980s and 2001 and demonstrated that in some parts of southern Cameroon the area of land previously in agriculture but currently in fallow was greater than the area of land previously in forest and currently under agriculture, in both the 1980s and 2001. Blaming shifting cultivators for deforestation is unhelpful. What is more important is to assist in developing best-bet alternatives with economic incentives for producers such as giving a price premium for goods produced without damaging the forest.

Theobroma cacao L. (Sterculiaceae) is a lower canopy tree, usually 4 – 8 m tall, native to south and central American forests. It is cultivated throughout the humid tropics for its cocoa beans, the raw product for chocolate. In the humid tropical forests of Cameroon, cacao is grown by more than 70% of farmers, predominantly under a forest canopy where upper-canopy shade trees, selected for their timber, fruits, nuts or medicinal products are retained. Yields are low and to improve livelihoods of smallholder farmers, methods to increase the yields are crucial for sustenance of this landuse.

There have been recent studies demonstrating the biodiversity benefits of shaded cacao systems, with foci on avian, primate and plant diversity and how to manage farms to optimize diversity. No studies have looked at effects of management in cacao on below ground biodiversity, although soil fauna play a major role in nutrient cycling in the tropics where fertilizer is rarely used (Norgrove 2007). In an agroforestry trial in southern Cameroon, Dibog *et al.* (1999) demonstrated that tree canopy cover was positively correlated with termite abundance and species richness and, furthermore, yield of the under storey tannia crop (*Xanthosoma sagittifolium* L.) was positively correlated with the abundance of soil-feeding termites, which were postulated to have a positive impact on soil physical properties. Norgrove (2007) found a negative correlation between cacao litter and earthworm surface casts found in quadrats, suggesting that earthworms play a major role in the breakdown of recalcitrant cacao litter.

It is hypothesized that if secondary forest cover were to decrease as economic development advances, cacao farms would play an increasingly important role as seed repositories and in harbouring the beneficial soil fauna, thus providing landscape-level benefits by impacting adjacent fallows through seed dispersal and soil faunal migration. However, these ecosystem processes might be affected by management, in particular fungicide application and changes in upper-canopy tree density and shade level.

Biodiversity considerations aside, cocoa yields need to be profitable otherwise farmers will resort to other land uses. Blackpod disease, caused by *Phytophthora* fungi, predominantly *P. palmivora* Butl. and *P. megakarya* Bras. & Griff, is the major biotic yield constraint worldwide. There are biological, cultural and chemical interventions to reduce blackpod. The use of bio-control agents, such as the soil mycoparasite ('*Trichoderma*') is in the development stage (Samuels *et al.* 2006). Cultural controls include phytosanitary harvests comprising the removal of diseased pods from the field to minimise inoculum. However, Norgrove (2007) demonstrated that even with fortnightly rigorous phytosanitary harvest, cocoa yields are negligible without spraying in *P. megakarya* infested areas. Reducing shade and increasing airflow in the understorey reduces blackpod, however, this would be likely to increase mired attacks, warranting the use of predominantly class I or II insecticides and therefore, is not recommended. Furthermore, apart from reducing canopy diversity and carbon stocks, soil faunal population (Dibog *et al.* 1999) decline when trees are removed from land use systems.

In West and Central Africa, most commonly used fungicide is copper oxide (Cu_2O) which is of the low toxicity i.e., Class III or IV (Matthews *et al.* 2003) but at rates much lower than manufacturers' recommendations (Norgrove 2007). Higher fungi-cide spray rates increase the yield differential over time compared with lower rates (Norgrove 2007). Furthermore, the use of copper oxides only could still permit the farmer to collect an organic price premium.

However, what might be the impact of increased fungicide input on ecosystem function? In temperate zones, long-term use of copper fungicides has had negative impacts on soil fauna and other non-target organisms (Bengtsson *et al.* 1992; Reinecke *et al.* 1997; Van Zwieten *et al.* 2004). We assessed the effects of different fungicide application rates (high, low and zero) on earthworms and termite population in cacao farms in southern Cameroon and compared this with secondary forest of the same age. We considered species richness, density and ecological classification as parameters. In addition, we assessed the role of earthworm casts in soil seed bank dynamics.

Material and methods

Field experiment

An experiment was established in Zoatoupsie approximately 10 km south of Mbalmayo in southern Cameroon (3° 51' N and 11° 27' E) and plots were established in farmers' cacao (*Theobroma cacao*) farms with upper canopy trees. Details of the general area are described in Norgrove (2007). The cacao had been abandoned for three years. Plots were selected for visual uniformity. The cacao was approximately 35-yr old and had been reportedly established after selectively felling secondary forest and a single food crop cycle without the use of agrochemicals.

The experiment was conducted in a replicated randomized block design with three treatments: (1) 'high concentration' Ridomil® plus 72 WP (active ingredients 600 g kg^{-1} Cu_2O and 120 g kg^{-1} metalaxyl i.e., $C_{15}H_{21}NO_4$) at 6.4 kg Ridomil® ha^{-1} yr^{-1} divided into eight applications (recommended application); (2) 'low' concentration' at 2.13 kg ha^{-1} yr^{-1} divided into eight applications. (3) zero or no spray. Also, an unsprayed secondary forest control (non-randomised) was used as a comparison for each block. The secondary forest control had reportedly been cleared for crop production 35 years previously at the same time as the cacao and left to regenerate. The experiment had three replicates. Plot size was 25 m x 25 m. The experiment was continued for four years.

Termites were sampled in December 2001, at the beginning of the dry season and at the end of the first year of treatment. Ten 5 x 5 m areas were sampled per plot, avoiding the edges. 1 man hour sampling time was used per segment. Within the sampling area, soil to 10 cm depth was randomly sampled, including surface litter. Furthermore, areas under dead wood, areas between tree buttresses, mounds, arboreal nests, and any runways on trees were sampled. Every species, but not every termite was collected so data are qualitative. Feeding classification follows Eggleton *et al*. (1995).

Earthworm sampling was conducted after four years of treatment in one 0.75 m x 0.75 m frame per plot using the formalin expulsion technique. Earthworm sampling was conducted at this period because earthworm cast production showed significant differences between treatments in the fourth year (Norgrove 2007). 5 l of 0.02% formalin solution was poured into the sampling area and earthworms were collected as they surfaced. After 30 minutes, a further 5 l of solution was added and earthworms collected thus sampling effort was 1 man hour per frame. Every individual was collected, therefore, estimates are quantitative. Earthworms were killed in 70% alcohol, counted and transferred to 4% formalin for preservation. Cast production had been monitored in this frame in the previous year (Norgrove 2007) and total production data were used to compare with species data.

Pot experiment

Soil and freshly voided earthworm casts were also sampled in all plots for seedbank analysis. Six pots without drainage holes were prepared with soil or casts from each plot of which four were planted with 50 seeds per pot of lettuce (*Lactuca sativa* L.), and chilli (*Capsicum annum* L.) and two were left for seedbank analysis. Pots were watered twice weekly. Emergent plants were counted and for the seedbank analysis identified to the species level.

Datasets were analysed using the GLM procedure in SAS v. 9.1 on untransformed data after normality checks. Regressions between earthworm densities by species were regressed against annual cast production of the previous year using PROC REG. Termite frequency refers to the percentage of segments in which the species was present.

Results

Termites

Less termite species were found in cacao than forest, but there were no differences between cacao treatments (Table 1). A full list of species found is given in Appendix Table 1. The three most frequently encountered species in the forest were the specialist soil feeders *Cubitermes fungifaber* (33% of segments) and *Basidentitermes maleleanses* (26.7% of segments) and wood / litter/ fungus feeder *Acanthotermes acanthothorax*, occurring in 30% of segments.

True soil feeders were the most frequently represented ecological grouping with eighteen species present. Four species were absent in cacao (*Crenetermes mixtus, Profastigitermes putnami, Apillitermes longiceps* and an *Apillitermes* sp.) and the rest occurred at less than 25% of the frequency of in the forest, the most frequent true soil feeder, *C. fungifaber,* occurring in only 1% of cacao segments. *Basidentitermes maleleanses* was the only species found commonly (13.3% of segments) in cacao. Of eight species of specialist wood feeders, *Nasutitermes nigeriannus*, *Microcerotermes maleleanses* and a *Nasutitermes* sp. were absent in cacao. However, two *Microcerotermes* species, *M. fuscotibialis* and an unknown sp. were encountered more frequently in cacao than in forest (33 and 24% of fragments respectively in cacao compared with 10 and 7% in forest). Overall mean encounter rates of special wood feeders were similar in forest and cacao.

Of seven species of more generalist wood / fungus eaters, *Pseudocanthotermes fuscotibialis*, *Pseudocanthotermes spiniger*, and *Protermes prorepens* were present exclusively in cacao. Of three soil / wood interface generalist feeders

Table 1. Termite species richness in secondary forest and adjacent cacao farms, pooled across fungicide treatments (N = 3 forest; N = 9 cacao). Numbers suffixed by different letters in the same column are significantly different at $P < 0.05$.

Ecosystem	# species	# genera
Forest	29a	20
cacao	19b	17

present, *Termes hospes* and an *Amalotermes* sp. were present exclusively in cacao.

Earthworms

Earthworm genera represented were: *Dichogaster; Eminoscolex; Eudrilidae* gen. nov.; *Ilyogenia; Legonodrilus; Malodrilus; Nematogenia* and *Rosadrilus* (Table 2). Adult densities were significantly (P < 0.05) higher in forest than any of the cacao systems. However, total densities (adult + juveniles) were not significantly different between forest and unsprayed cacao, yet those in sprayed cacao treatments were significantly lower (Fig. 1).

Comparing forest with cacao systems, there was a major shift in earthworm communities. When considered by genera and feeding classification, there were no significant effects of land use system on the densities of the endogeic taxa *viz*., *Ilyogenia* sp. and *Legonodrilus* sp., however, *Nematogenia* sp. was present in all forest plots but only one individual was found in the cacao. Conversely, *Dichogaster adjelana* was present in cacao but absent in forest (Fig. 2). *Eminoscolex lamani* and *Rosadrilus camerunensis* had the highest adult densities in forest. *R. camerunensis,* the only anecic species identified, declined to only 20% of the forest densities in cacao and was eliminated in the high spray treatment.

Table 2. List of genera and species, where determined, in forest and cacao systems, in Zoatoupsie, southern Cameroon.

Family and species	Feeding strategy
Ocnerodrilidae	
Nematogenia sp.	Endogeic
Ilyogenia sp.	Endogeic
Eudrilidae sub family Eudrilinae	
Rosadrilus camerunensis Cognetti	Anecic
Eminoscolex lamani Michaelsen	Epigeic
Malodrilus buarensis Michaelsen	Epigeic
Eudrilidae sub family Pareudrilinae	
Legonodrilus sp.	Endogeic
Eudrilidae *gen. nov.*	Endogeic
Acanthodrilidae sub-family Benhamiinae	
Dichogaster (Dt.) adjelana Michaelsen	Endogeic
Dichogaster(Dt.) sp.	Epigeic

Of the epigeic earthworms, *E. lamani* had the highest density in forest but was absent in cacao. Yet the response of other epigeic species was divergent with the remaining genera having a higher density in cacao systems.

Adult population data of the anecic and endogeic species were regressed against cast production. The density of *Rosadrilus camerunensis* was significantly (P = 0.0022) and positively correlated (R^2 = 0.62) with the annual cast production per frame whereas *Ilyogenia*, *Legonodrilus* and *Nematogenia* were neither correlated nor explained by any residual variation.

There were significant (P < 0.05) differences in seed bank densities among various soil classes. If forest soil is considered at 100, forest casts were 71, cacao soil was 31 and cacao casts 75. The number of seedbank species was significantly (P < 0.01) higher in casts (6.4 per plot) than in soil

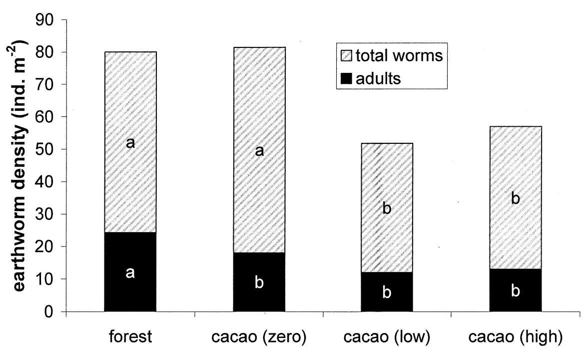

Fig. 1. Density of adult and total (adult + juvenile) earthworms in forest and high, low and zero fungicide treated cacao in southern Cameroon. Different letters between columns of the same colour indicate significant differences at P < 0.05. N=3.

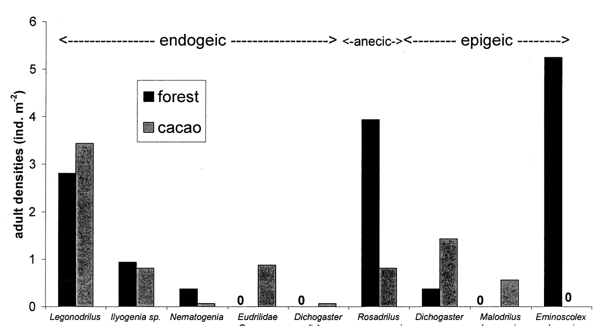

Fig. 2. Densities of adult earthworms in forest and cacao, separated by species and feeding category. N = 3 for forest and N = 9 for cacao.

(4.3 per plot). Germination for both of the planted species was significantly ($P < 0.0001$) higher on casts (57%) than on soil (37%).

Discussion

Eggleton *et al.* (2002), looking at chronosequences of land use cover across southern Cameroon found that cocoa harboured approximately half the number of species of secondary forest. Here we found less difference between species richness of cacao and forest systems and were also, crucially, comparing systems of the same age. However, we noted the loss of true soil feeders in the cacao environment and the general tendency for specialists to be replaced by generalists. Deblauwe *et al.* (2007) reported that soil feeders are more strongly affected by disturbance, as they need stable conditions given their energetic limitations (Eggleton *et al.* 2002). One of these, the humifagus afro-tropical *Cubitermes* is found in both savannahs and forests (Roy *et al.* 2006) and is one of the main mound builders (Dejean & Ruelle 1995). As these mounds are often inhabited by other species, a reduction of *C. fungifaber* has knock-on effects on the diversity of other genera.

In general, the elimination of the dominant epigeic species *Eminoscolex lamani* in the cacao is not surprising as it is likely to be negatively affected by the reduction in litter quality given the recalcitrant nature of cocoa litter. Norgrove (2007) reported significant differences in copper concentrations among cocoa litter under different spray treatments, with (mean ± S.E.) 11.8 ± 1.7 mg kg^{-1} in the zero spray treatment, 15.7± 3.9 mg kg^{-1} in the low treatment and 43.2 ±7.8 mg kg^{-1} in the high spray treatment, which might negatively affect epigeics and anecics. The presence of *Dichogaster adjelana* in the cacao but absence in the forest is surprising. It is a native Cameroonian worm and has not been reported from outside Cameroon. Birang (2004) reported its occurrence in annual cropped fields but not in native forest in southern Cameroon. It is a large worm attaining 8 cm length, probably a polyhumic endogeic living in the uppermost soil layer. Speculating on its absence in forest, it may be negatively affected by competition with the more abundant anecic *R. camerunensis*. While the *Dichogaster* are probably native to Africa (Blakemore 2003; Csuzdi 1996), they can be peregrine and invasive. For example, Erséus *et al.* (1994) reported the presence of *D. saliens* and *D. bolaui* in Sweden and later, Csuzdi *et al.* (2008) demonstrated that *D. bolaui* became common in Israel and Hungary. Norgrove *et al.* (2008) reported that the peregrine *D. saliens, D. modiglianii* and *D. annae* were present in association with alien invasive plants in the Cameroon highlands yet were absent in native savannah systems (Norgrove *et al.* 2008).

Adult population densities of the deep burrowing endogeics *viz.*, *Ilyogenia* sp. and *Legonodrilus* sp. were not different between cacao and forest, given less impacts on the soil resource in deeper soil layers. Yet, densities of the anecic *R. camerunensis* were greatly reduced in cacao and eliminated in the high spray treatment. Norgrove (2007) showed that earthworm cast production at this site was higher in the forest than in the cacao systems in all four years of the experiment, with the high spray treatment having lower cast production (4.6 Mg ha^{-1}) than either the zero spray (19.2 Mg ha^{-1}, P diff = 0.017) or the low spray (12.1 Mg ha^{-1}, P diff= 0.09) in the fourth year. The correlation between cast production and population density of this large-bodied worm strongly suggests that it is the major cast producer in the system and thus deserves further research. In a comparison of cast production in villages in southern Cameroon, the highest cast production occurred where *R. camerunensis* was reported as present the most times and the lowest production occurred in villages where it was reported as absent (recalculated from Birang *et al.* 2003; Birang 2004). Norgrove (2007) reported that casts collected in the high fungicide application plots, where *R. camerunensis* was not found, had a higher percentage of clay and were more similar in texture to soil from deeper layers. This again suggests that casts in these plots were of the deeper burrowing endogeic worms rather than the anecic *R. camerunensis*.

The endogeic *Ilyogenia* sp. may have a highly localized distribution as it has been reported from this area (Birang 2004) but did not occur in samples 80 km to the north or to the more forest dominated systems 80 km to the south, although it was found locally in system of varying disturbance. Interestingly, the *Nematogenia* sp. was found commonly in forest but rarely in cacao. In other work conducted in southern Cameroon (Birang *et* *al.* 2003) and the western Highlands (Norgrove *et al.* 2008), the sole *Nematogenia* representative, *N. panamaensis* was found only in short fallow invaded systems and not in forest, contradicting the current results and suggesting an invasive status similar to *O. occidentalis*, however, it may be sensitive to spraying.

Results from the soil seedbank study highlighted the importance in maintaining cast voiding species in derived systems. Results suggest that the differences in diversity are due to improved germination percentages of seeds in casts rather than that more species require to be voided by earthworms for germination.

In conclusion, we report some reductions in termite species diversity, earthworm activity and species richness in cacao agroforests. This was accompanied by a shift from specialist to generalist feeders in termites. Earthworm densities were reduced with a reduction of the anecic, major cast producer in cacao and its elimination in the high spray treatment. Presumably due to contaminated litter. Such impacts may be minimized using improved narrow angle cone nozzle sprayers in fungicide treatments (Bateman 2004) and possibly adding mulch to the ground litter to improve litter quality.

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Appendix Table 1. Termite species found in cacao and forest systems in southern Cameroon with feeding category. S - soil, W - wood, L - litter, F - fungi, R - roots (following Eggleton *et al.* 1995).

Termitinae, Termes group	
Pericapritermes urgens	S
Termes hospis	S/W
Thoracotermes macrothorax	S
Termitinae	
Apillitermes longiceps	S
Apillitermes sp.	S
Basidentitermes maleleanses	S
Crenetermes mixtus	S
Crenetermes fungifaber	S
Crenetermes sp.	S
Cubitermes fungifaber	S
Cubitermes heghi	S
Fastigitermes jucundus	S
Foraminitermes rhinocerus	S
Microcerotermes fuscotibialis	W
Microcerotermes sp.	W
Microcerotermes maleleanses	W
Microcerotermes arborum	W
Proboscitermes tubiliferus	S/W
Procubitermes undulans	S
Procubitermes sp.	S
Profastigitermes putnami	S
Nasutitermitinae	
Nasutitermes arborum	W
Nasutitermes nigeriannus	W
Nasutitermes sp.	W
Macrotermitinae	
Acanthotermes acanthothorax	WL F
Microtermes pusillus	W F
Odontotermes sp.	W F
Protermes prorepens	W F
Pseudocanthotermes fuscotibialis	WL F
Pseudocanthotermes spiniger	WL F
Sphaerotermes sphaerothorax	W
Synacanthotermes heterodon	W R
Apicotermitinae	
Adaiphrotermes sp.	S
Adaiphrotermes sp2	S
Amalotermes sp.	S/W
Astalotermes sp.	S