INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES
IJPAES
Volume-5, Issue-1, Jan-Mar-2015 Coden: IJPAJX-USA Copyrights@2015 ISSN-2231-4490
Received: 20th Dec-2015 *Revised: 24th Jan-2015* *Accepted: 24th Jan-2014*
Research article

RESPONSE OF SOIL FEEDING TERMITES TO THE EXPERIMENTAL INPUT OF ORGANIC MATTER

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ABSTRACT: Trophic chains are controlled by the availability of resources and predation pressure. Therefore, this study was conducted to evaluate the influence of the experimental input of organic solutions on termites' assemblies in the soil. The experimental design consisted of two blocks at each site, with each block consisting of: (a) two plots treated with *Ch. Odorata* , another two plots treated with *A. cordifolia*, while the control plots received no treatment, for termites' inventory (b) six plots similar to (a) for the determination of carbon and nitrogen. The results obtained showed that the response of termites to the nutrient solutions was strongly influenced by the physical environment and seasonality. In contrast to the expected results, the treatments had no significant effect on the density and diversity of termites, although the species richness and diversity of termites were higher in plots subjected to treatment.

Key words: Experimental, input, soil feeding termites, organic matter

INTRODUCTION

Trophic chains are controlled at the lower levels by the availability of resources while at the higher levels, they are controlled by predation pressure [1, 2, 3, and 4]. In soil, quality and quantity of food resources structure and determine the activity of soil organisms. In springtails and earthworms it has been shown that the availability of nitrogen and carbon resources, determine the development of the community [5 and 6]. Earthworms and termites are the two most important groups of organisms in tropical soils; earthworms represent 49 g/m^2 and termites 1.9 g $/m^2$ in Lamto, Ivorian savanna [7]. Studies in USA have shown that earthworms increased mineralization of soil carbon [8] compared to humivores termites whose activities contribute to the stabilization of soil organic matter [9]. The soil feeding termites represent the majority of termite species and are found in all tropical soils [10]. Some of these termites live in the soil, in diffuse constructions, while others build epigeal nests, biomasses up to 200 g $/m^2$ [11]. This density implies that much of the upper soil is altered by passage through the "trickling filter" that constitutes their digestive tract. The organic matter intake is given to the ecosystem via faeces included in termite mounds and galleries forming the termitosphere [12].

Their food substrate is humified organic matter presents in soil. Humus ingested by soil feeding termites consists predominantly of aromatic compounds resulting from the degradation of lignin and tannins [13]. Cellulose degradation is based on hydrolysis of poly- and mono-saccharide using hydrolases (cellulases, hemicellulases, etc.), that of lignin depends on various strategies where more hydrolases are not necessarily present. [14] Showed that there was absence of cellulolytic, hemicellulasic and amylasic enzymes in soil feeding termite species while these enzymes are present in the other diets. This lack of hydrolytic enzymes is not limited to carbohydrates; in fact, [13] showed the low presence of enzymes in the degradation of lignin in six species of soil feeding termites. It appears that the degradation of the humified organic matter goes through an alkaline hydrolysis step at the posterior gut [15] followed by mineralization and / or microbial fermentation [16]. The soil feeding regime is in the evolving context of termites, the most recent diet providing access to an abundant resource but lower quality [17]. This diet promotes specialization which would explain the exceptional biodiversity of this functional group (about 1,100 species in 2300 described) [18, 13]. It is in the humid forests of Africa, rich in organic matter, that the highest species richness was observed [19].

The amount of soil ingested by soil feeding termites varies between 2.76 and 9.1 g / d [20]. Species appear to fall into micro niches along a humification gradient that goes from much degraded wood to very humified organic matter [19]. [21] Suggests that this distribution of soil feeding termites is based on the size of the species, where small species living in the upper strata rich in organic matter, larger species lying in the deeper layers of soil.

From the experiments on soil fauna, [22], [6] and [23] found that bacteria, fungi as well as saprophagic invertebrates such as earthworms are controlled by the amount of available resource. [6] Observed an increase in the biomass of earthworms in plots in response to glucose intake. But studies on the influence of the experimental input of organic matter on soil feeding termites assemblies has not documented. The objective of this study was to determine if the addition of organic matter in the soil modify termite assemblies. We hypothesized that: regular intake of organic matter should increase the local density of soil feeding termites as it is assumed that their density is limited by the available organic matter and exploitative competition. Specifically we sought to:

- Assess the influence of experimental organic matter input on carbon and nitrogen from the soil;
- Assess the influence of the experimental input of organic matter on abundance, species richness and diversity of termites.

MATERIALS AND METHODS

The experiments were conducted at three sites; Madong (Atlantic Coastal Forest), Ongot (mixed forest) and Mbong Sol (semi-deciduous forest) respectively. These sites are characterized by a humid equatorial climate (see Table 1, Figure 1).

Table 1: Locations and characteristics of the study sites

location (Ecosystem)	Annual rainfall and mean temperature	Coordinates
Madong (Evergreen Atlantic forest)	3030 mm, 23°C	3°27.40 N, 10°74.84 E,
		496m alt.
Ongot (Mixted forest)	1570 mm, 21°C	3°51,76 N, 11°23,07 E
		760 m alt.
Mbong Sol (Semi-deciduous forest)	1530 mm, 23°C	4°39.49E, 12°24.37 E,
		643m alt.

(Data from 2008)

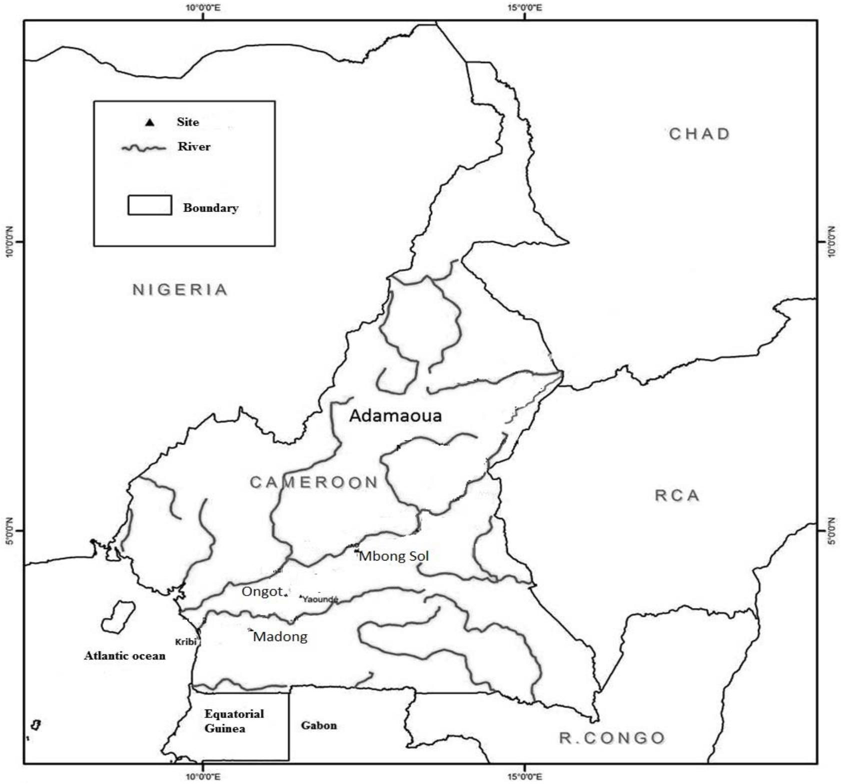

Figure 1: Study sites

Organic matter inputs

For this study, two plants were selected

Chromolaena odorata (L.)King and Robinson (Asteraceae)

Ch. Odorata was chosen because previous studies in Cameroon have shown that this plant improves soil properties. Indeed in southern Cameroon, [24] showed that *Ch. Odorata* has a beneficial effect on the concentration of exchangeable K in sandy and sandy-clay soils by comparing fallow dominated by *Ch. Odorata* and non-fallow dominated land. Also this plant maintains soil organic matter of multi-year fallow as levels comparable to that of a wet forest soils [25] or tree fallows [26].

Alchornea. cordifolia (Schumach. & Thonn.) Müll.Arg. (Euphorbiaceae)

A. cordifolia is widespread in the savanna and secondary forests of tropical Africa. This shrub can be found on swampy or dry sites. It thrives in acidic soils. It behaves as a pioneer in many fields in the tropics. It is regarded as a medicinal plant. In Lower Congo, this plant is known for its ability to increase soil fertility because it enriches acidic soils with calcium [27].

Experimental device

At each site, the experimental setup consisted of two blocks. Each block consists of: (a) six plots of 1.50m x 1m, 1m remote for sampling of soil fauna; two plots treated with *Ch. Odorata*, another two plots treated with *A. cordifolia,* while the control plots received no treatment, (b) consisting of six plots similar to (a) for the determination of carbon and nitrogen. In each plot, eight tubes in PVC were implanted in the ground in two rows of four; both rows of tubes are distant from 50 cm. The tubes on the same row were spaced out by 30 cm each. Each tube measured 13cm with an inner diameter of 2.8cm and was put into the ground at 3cm of depth. In treated plots, each tube received 75 ml infusion per month.

Principle: Experimental organic matter intakes were prepared by infusing leaves of two plants solutions *Chromolaena odorata* and *Alchornea cordifolia*. Solutions of organic matter are brought regularly for six or nine months on plots after which the treated soils and control soils are harvested for termites' inventory. For the preparation of solutions, 73 g of leaves of a particular plant species, previously dried in an oven at a temperature of 40°C for 12 hours, is brought to boiling in 3 liters of water for 1 minute. An evaluation of soil organisms particularly termites was performed after 6 and 9 months. Eight cores of soil 8 cm in diameter and 10 cm deep were taken from each plot at the location of the tubes. They were wet sieved on two sieve, 2mm and 0.8mm respectively. Whole macrofauna harvested were kept in tubes containing 80% ethanol. To estimate the concentrations of nitrogen and organic carbon two samples per plot were collected at a depth of 10cm

Determination of total nitrogen and organic carbon

Total nitrogen was determined by the Kjeldahl method and the organic carbon was assayed by oxidizing potassium dichromate.

Data Analysis

Data were recorded in an Excel spreadsheet. The number of termites (log Ln $(N + 1)$ transformation) from the different plots were compared by factorial analysis of variance followed by Tukey HSD test at 5% significant level, using STATISTICA ver. 9.0 software. Spearman correlation coefficient was used to assess the degree of connection between the concentrations of soil carbon (g / kg) and nitrogen (g / kg) and the number of termites. The Shannon index was used to assess the diversity.

RESULTS

The concentrations of nitrogen and carbon in *Ch. Odorata* and *A. cordifolia* leaves are shown in Table 2. On average ($N = 2$), the infusions of *A. Cordifolia* and *Ch. Odorata* contained 9.17 g C / l , 1.46 g N / l and 8.7 g C / l , 1.52 g N / l respectively.

Influence of the treatments on carbon and nitrogen and the soil

Carbon

The carbons in plots were subjected to a factorial analysis of variance. Neither treatment (2 df, $F = 3.28$, $p = 0.049$) and duration of treatment (1 df, $F = 0.87$, $p > 0.05$) significantly changed concentration of carbon in soil; ecosystems and duration of treatment has almost no influence ($N = 8$ for each ecosystem) (see figure 3) (2 df, $F = 2.89$, p 0.05). But one significant interaction was noted:

Between the ecosystem and the type of treatment: analysis of variance (ANOVA) showed that the effect of these nutrient solutions on soil carbon depended on the ecosystem (4 df, $F = 5.48$, $p < 0.01$); in the Atlantic Forest, treatment with *A.cordifolia* and *Ch. odorata* caused a decrease in the carbon content, while in the other two forests it caused a slight increase (Tukey test, 63 df, $p < 0.05$) (figure 2).

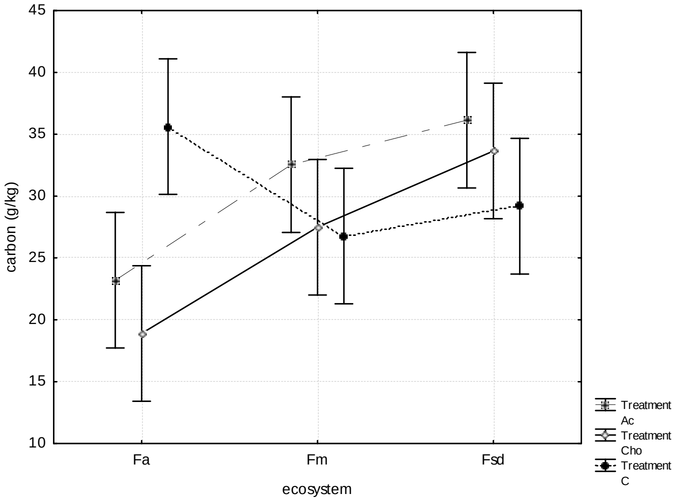

Figure 2: Carbon concentrations (g/kg) in the treated and control plots depending to ecosystem (Ac : *Alchornea cordifolia,* Cho : *Chromolaena odorata,* c : control)

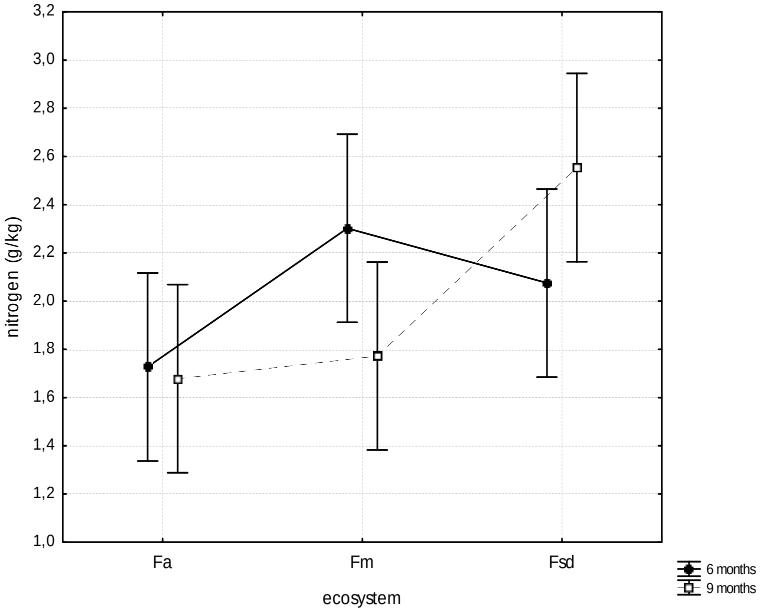

Figure 3: Nitrogen concentrations (g /kg) in the ecosystem depending on the duration of treatment (Fa: Atlantic Forest, Fm: Mixed Forest, Fsd: semi-deciduous forest)

Table 2: Nitrogen and carbon concentrations (g/kg) on leaves of *Ch odorata* and *A. cordifolia*

Leaf	Nitrogen (g/kg)	Organic Carbon (g/kg)
Chromolaena odorata	0,5 19 ±0,11	45,87 ± 1,79
Alchornea cordifolia	0,48 ±0,04	44,20 ± 2,82

Table 3: Nitrogen concentrations (g/ kg) (mean ± standard deviation) depending on the treatment and ecosystem, with the results of the Tukey's test (N = 8 per treatment)

Locality	*A. cordifolia*	*Ch. Odorata*	Control
Atlantic forest	1,98 ± 0,31 ns	1,73 ± 0,25 ns	1,26 ± 0,42ns
Mixed Forest	2,36 ± 0,64 *	2,19 ± 0,52 *	1,55 ± 0,15 ns
Semi-deciduous forest	3± 0,81**	2,63 ± 0,73**	1,3 ± 0,13 ns

** = Highly significant difference p <0.01; * = Significant difference, p <0.05; ns = non-significant difference, p> 0.05

Nitrogen

The nitrogen concentrations were subjected to the same analysis; these concentrations are significantly different according to the treatment (2 df, F = 50.74, p < 0.01) and the ecosystem (2 df, F = 17.36, p < 0.001); but they did not vary during the observed period (1 df, F = 0.004, p > 0.05). Nitrogen concentrations were higher in plots subjected to treatment than in the control plots (Tukey test, 36 df, P < 0.01). There were significant interactions between both treatments and ecosystems (4df, F = 4.56, p < 0.01) and the other between period and ecosystems (2 df, F = 10.32, p < 0.01) (Table 3, figure 3).

Abundance

The analysis of variance showed that the addition of organic matter by solutions (2 df, F = 0, 23, p > 0.05) and the type of vegetation have no effect (2 df, F = 2.55, p > 0.05), but the period is the only variable affecting the abundance of termites, the average number were (1.94 ± 1.72) at 6 months and (1.59 ± 1.49) at 9 months (1ddl, F = 4.23, p < 0.05). However number of termites was lower in the control (1.69 ± 1.63) than in plots treated with solutions based on *Ch. odorata* (1.77 ± 1.59) and *A. cordifolia* (1.83 ± 1.64). These averages are statistically similar (Tukey test, 252 df, p > 0.05) (Figure 4a). Furthermore, the effects of different treatments on termite assemblages were assessed in each ecosystem. The results showed that, abundance of termites was variable depending on the treatment according to the ecosystem (279 df, F = 2.81, p < 0.05) (Table 4).

Species richness

Species richness recorded in each plot according to the treatment of the ecosystem and the time was subjected to analysis of variance; this analysis revealed that species richness in plots subjected to treatment was variable depending on the type of ecosystem (2 df, F = 9.89, p << 0.05) (Table 5). The supply of organic matter and the period did not affect it. However, the average species richness (± standard deviation) was lower in the control plot (0.87 ± 0.78) than in plots treated with *A. cordifolia* (1.04 ± 1.00) and *Ch. odorata* (1.03 ± 0.91) (Tukey test, 279 df, p > 0.05) (Figure 4c).

Diversity

In each ecosystem, species diversity observed in the treated plots was compared with that observed in the control depending on the period; the results showed that species diversity in plots varied depending on the ecosystem (2 df, F = 2.98, p < 0.05) (Table 6). In the Mixed and Semi-Deciduous Forests the highest diversity was found in plots subjected to *A. cordifolia*. Diversity index obtained in plots with *A. cordifolia* in the semi-deciduous forest was the highest of all plots. It was statistically different from that observed in the same plots in the Atlantic Forest (Tukey test, 27 df, p < 0.05) (Table 6). In general, the addition of organic matter in the soil by these solutions did not affect the diversity although diversity indices obtained in plots *A. cordifolia* (0.48 ± 0.25) and *Ch. odorata* (0.40 ± 0.13) were higher than the control plot (0.35 ± 0.14) (Tukey test, 2 df, p > 0.05) (Figure 4b).

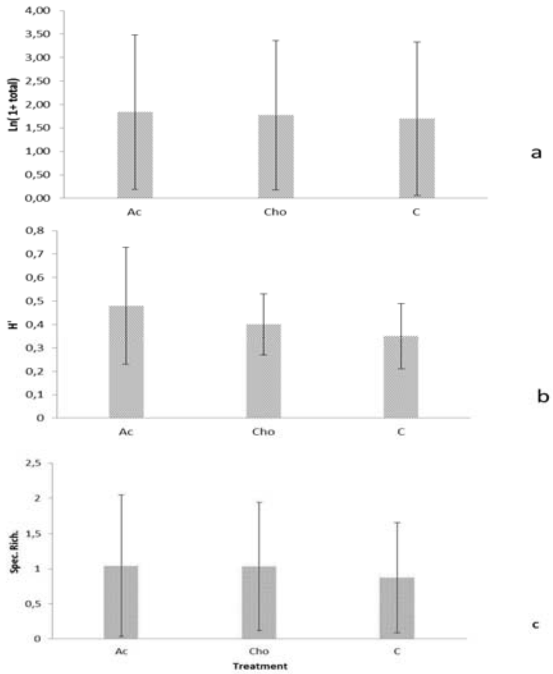

Figure 4: Abundance (a), diversity (b) and specific richness (c) of termites (Mean ± standard deviation) depending on the treatment. (Ac: *Alchornea cordifolia*, Cho: *Chromolaena odorata*, c: control, N= 96)

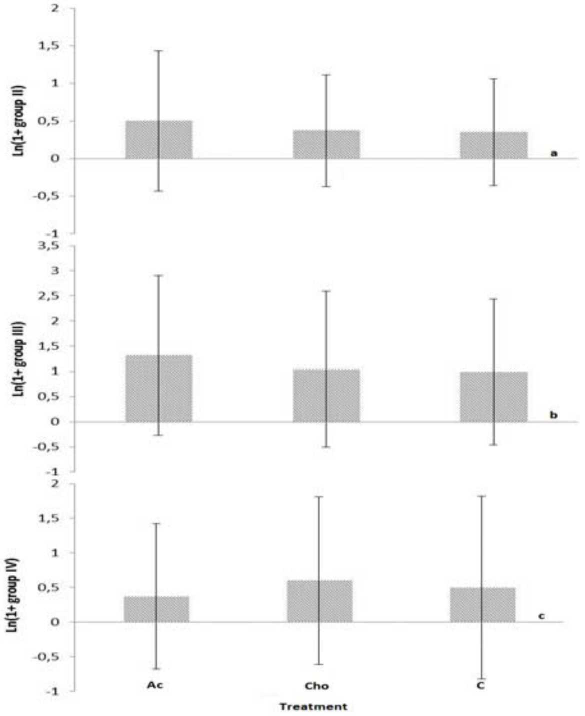

Figure 5: Termites abundances (mean ± standard deviation) of groups II (a), (b) III and IV (c) depending on the treatment. (Ac: *Alchornea cordifolia*, Cho: *Chromolaena odorata*, C: control, N = 96)

Figure 6: Area receiving monthly an infusion of *C. odorata*, Mbong Sol (observation after 6 months)

Table 4: Abundances (mean± standard deviation) (Ln (1 + total)) of termites depending on treatment and ecosystem, with the results of the Tukey's test (N = 32 per treatment)

Locality	*A. Cordifolia*	*Ch. odorata*	Control
Atlantic forest	1 ± 1,62*	1,92±1,65ns	1,56 ±1,95ns
Mixed Forest	2,48±1,54*	1,57±1,7ns	1,71±1,44ns
Semi-deciduous forest	2,03±1,45ns	1,82±1,44ns	1,80±1,5ns

** = Highly significant difference p <0.01; * = Significant difference, p <0.05; ns = non-significant difference, p> 0.05

Functional Groups

Termites collected were classified into three functional groups [28] termitinae wood feeding (Group II), wood-soil feeding (group III) and true soil feeding (Group IV). For all functional groups, input of organic matter by infusions had no significant influence on their abundance in plots (Tukey test, 279df, $p > 0.05$); but termites of groups II and III tended to be more abundant in the plots subjected to treatment compared to the control (Figure 5a and 5b).

The functional groups were significantly influenced by the type of ecosystem (Tukey test, 2 df, $p << 0.05$) (figure 6). However, the period had a variable effect across groups. The abundance of termites groups III, varied during the observation period (2 df, $F = 4.77$, $p < 0.05$) in contrast to Groups II and IV. No interaction between factors was observed by the analysis of variance.

Table 5: Specific richness of termites (mean ± standard deviation) based on treatment and ecosystem, with the results of Tukey's test (N = 32 per treatment)

Ecosystem	*A. cordifolia*	*Ch. Odorata*	Control
Atlantic forest	0,4 ± 0,61ns	1, ± 0,81ns	0,62±0,70ns
Mixed Forest	1,40 ± 1,10**	0,93± 0,98ns	0,96 ± 0,73ns
Semi-deciduous forest	1,31 ± 0,93**	1,15± 0,95*	1,03± 0,86ns

** = Highly significant difference p <0.01; * = Significant difference, p <0.05; ns = non-significant difference, p> 0.05

Table 6: Specific diversity of termites (mean ± standard deviation) based on treatment and ecosystem, with the results of the Tukey's test (N = 4 per treatment)

Ecosystem	*A. cordifolia*	*Ch.odorata*	Control
Atlantic forest	0,26 ±0,19ns	0,44± 0,1*	0, 25± 0,19ns
Mixed Forest	0,68 ± 0,17*	0,32 ± 0,11ns	0,40± 0,1ns
Semi-deciduous forest	0,5± 0,23ns	0,44± 0,16ns	0,39± 0,06ns

** = Highly significant difference p <0.01; * = Significant difference, p <0.05; ns = non-significant difference, p> 0.05

Correlations

The degree of connection between the number of wood-soil feeding (group III), soil feeding (Group IV) and nitrogen and carbon concentrations (g/kg) in the plots were evaluated; the results showed a negative correlation between the abundance of soil feeding (r = -0.13), wood-soil feeding termites (r = -0.16) and the carbon concentration (g/kg) in the plots. However, positive correlations were observed between the number of groups III (r = 0.06) and IV (r = 0.11) and nitrogen concentration (g/kg) in plots. But in both cases, the observed values were not significant (p 0.05).

DISCUSSION

Species richness and abundance of termites

The main aim of this experiment was to manipulate resources assuming that the density of the organisms in the soil principally soil feeding termites may grow in response to an additional nutrient. Knowing that saprophagic soil fauna depends mainly on the availability of organic matter, we expected an increase in abundance of soil feeding termites in plots subjected to treatment compared to the control plots. In the present state of our knowledge, it is not possible to formulate a hypothesis about the change in species richness and abundance of termites. Numbers of termites in the plots was statistically independent of treatment received. But a trend towards increasing of soil feeding termites in plots subjected to treatment was observed (p = 0.08). It occurred only in plots receiving treatment with infusions of *Ch. odorata* (Figure 5c); The same trend was observed in species richness and diversity in the global plots subjected to treatment (Figure 4). Standard deviations much larger than the average, related to aggregative spatial distribution of termite, prevent significant results; in Figure 5, the standard deviations would "crash" the graph.

However we found that the response of termites to nutrient solutions varied in terms of ecosystems; in fact in the Atlantic Forest Coast, the number of termites observed in plots under *A. cordifolia* was lowest when in Mixed and Semi-Deciduous Forests termites were more abundant in plots subjected to this treatment. Environmental conditions (abiotic and biotic) profoundly changed depending on the site. The results obtained from manipulation of food resources from a locality remain ambiguous and therefore contribute little to the understanding of forces structuring community of decomposers. Control of an environment on assemblages of termites in soil resources is not simple. It results from a combination of factors including biotic and abiotic characteristics of the habitat.

Carbon

In the Atlantic Forest, carbon concentrations were lower in the treated plots compared to the control, while in the Mixed and the Semi-Deciduous Forests carbon concentrations in the treated plots were similar to the control.

In the Atlantic Forest site, the contents of carbon lower in the treated plots compared to the control are consistent with the expected result; indeed respiration is generally considered as a good measure of microbial activity.

Adding infusions in plots under *A. cordifolia* and *Ch.Odorata* had a positive influence on microbial respiration in this site; breathing would be higher in these plots which probably reflect the *priming effect* of these solutions on microbial activity and mineralization of soil organic matter. Such solutions appear to stimulate the oxidation of organic matter in the soil. These results are in agreement with those of [29] and [6] who observed an increase of microbial biomass followed by reduction of the organic matter due to the supply of glucose and nutrients in the medium. Carbon concentrations in the treated plots of Mixed and semi-deciduous Forests were similar to those of the control. In these sites a high activity of earthworms (abundance of fresh worm castings) was observed in treated plots (Figure 6). The activity of earthworms resulted in the production of biogenic structures, yet these organisms are known to produce large amounts of mucus. This mucus increases soil water holding capacity which changes the characteristics of the habitat [30]. The habitat changes by earthworms via the production of fresh biogenic structures and mucus appear to have thwarted mineralization of soil organic matter. The activity of earthworms significantly influences other soil organisms, due to indirect interactions through habitat alteration and directly through the exploitation of food resources. In temperate zones it has been shown that disruption of the soil by earthworms reduced the density of Oribatida [31, 32, 33 and 34] so that in the tropics, their activity appears to be harmful to termites [7].

Nitrogen

Both infusions increased significantly the nitrogen content compared to the control, but the duration of therapy had no influence. The nitrogen enrichment appeared to be higher with *A. cordifolia* than *Ch. Odorata,* but the difference is not significant. Increasing the nitrogen content in the parcels subjected to treatment is probably due to the immobilization of a portion of the nitrogen in bacterial biomass, which is in consistent with observations from [6]. This increase is more pronounced in Mixed and Semi-Deciduous Forests than Atlantic forest. The fact that the nitrogen content did not increase further after nine months compare to six months may be explained by nitrogen translocation by fungi; but our results were contradictory. Nitrogen inexplicably increased after nine months compare to six months in Semi-Deciduous forest while it declined in Mixed Forest.

Our study showed that supplementation of nitrogen on some level seems beneficial to soil organisms because species richness and diversity in *A. cordifolia* and *Ch. Odorata* plots were higher than the values observed in the control although observable differences were not always statistically significant. More non-significant, positive correlations observed between concentration of nitrogen and the abundance of soil feeding and wood-soil feeding termites indicate that these organisms are dependent on the availability of nutrients in the environment. This is consistent with studies [35] who observed that species richness and abundance of saprophagic fauna and even those of their predators were high in the sites receiving low doses of nitrogen.

Functional groups

Species of termites consume wood and litter during different stages of decomposition and humification and most termites' species are considered as soil feeding [36]. The supply of nutrients from solutions based on *Ch. Odorata* and *A. cordifolia* showed that soil feeding (Group IV) and wood-soil feeding (group III) are the most represented compared to wood feeding (group II) independent to the type of plot; this demonstrates that termites of groups III and IV seem to be more dependent on carbon and nitrogen from the substrate they exploit due to the fact that atmospheric nitrogen fixation in these organisms is low [37].

Period

We observed a gradual decrease in termites' abundance versus time; we found that species richness and abundance of termites in *A. cordifolia* plots after nine months were lower than those of the previous period (6 months). This would result from the influence of seasonality. Sampling of nine months took place during the long rainy season during which the abundance and species richness of termites in soil decreased [38].

CONCLUSION

This study showed that the response of termites to the nutrient solutions was strongly influenced by the physical environment and seasonality. But in contrast to the expected results, no treatment had significant effect on termites' density and diversity, although the specific richness and abundance of termites were higher in plots subjected to treatment. These treatments significantly influenced the soil nitrogen concentration. Furthermore the soil feeding termites were most abundant in plots.

ACKNOWLEDGEMENTS

We thank the Cameroon Oil Transportation Corporation (COTCO) for supporting the first author with a grant. We thank Prof. Mvondo Ze (University of Dschang, laboratory of pedology) for his advice in the soil analyses and Dr. Luc Dibog (IRAD) for his assistance in the identification of the termite and the access to the IRAD termite collection. We thank also Lynn Beleuk and Obi for valuable comments on an earlier version of the manuscript.

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