

NITROGEN FIXATION IN NASUTITERMES
IN CENTRAL AMAZONIA

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SUMMARY

Twenty one species of termites were collected from their nests in areas of pasture, secondary forest and primary forest at a cattle ranch in Central Amazonia, Brazil. They were tested immediately for nitrogen-fixing (acetylene-reducing) activity. The highest activities (more than 100 nmol C₂H₄ g dry weight⁻¹ h⁻¹) all occurred in the genus *Nasutitermes* collected in the pasture. *Nasutitermes* is known to be the commonest genus in the area. Acetylene-reducing activity associated with *Nasutitermes* spp. collected from the same nests on different occasions was very variable, and activity decreased with time after removal of the termites from the nests. Decapitation of the termites stopped activity immediately. The possible role of nitrogen fixation associated with *Nasutitermes* in the nitrogen cycle in Central Amazonia is discussed.

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RESUMEN

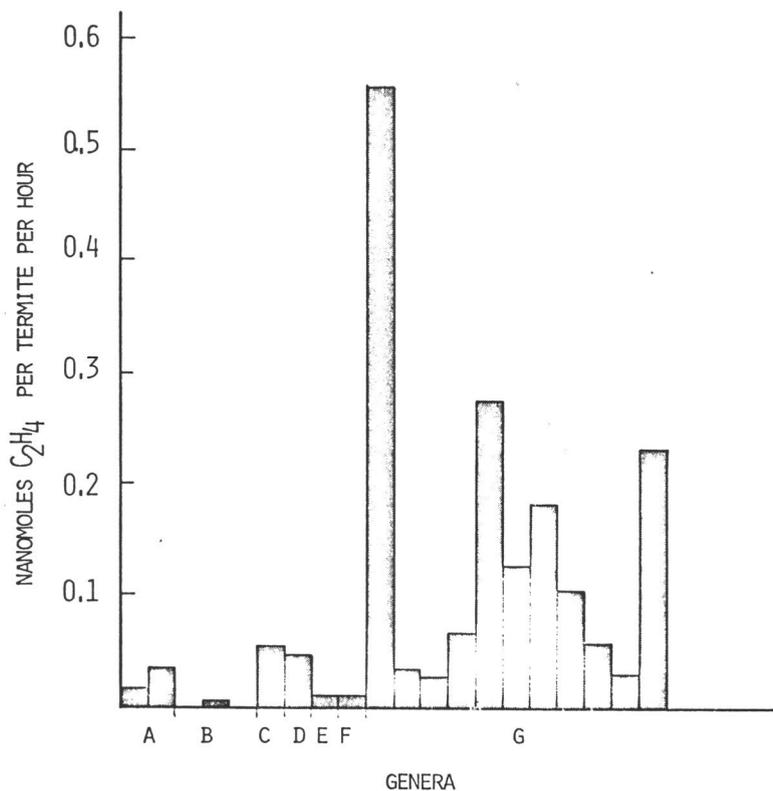
Fijación del nitrógeno en *Nasutitermes* de Centro Amazonia

Veinte y una especies de termitas fueron colectadas de sus nidos en áreas de pastos, de selva secundaria y de selva primaria en un rancho de Centro Amazonia, Brasil. Fueron testadas inmediatamente para la actividad de fijación del nitrógeno (reducción del acetileno). Todas las más altas actividades se encontraron en el género *Nasutitermes* colectado en pastos. *Nasutitermes* es el género lo más común en el área. La actividad de reducción del acetileno asociada con *Nasutitermes* sp. colectado del mismo nido en varias ocasiones fué muy variable, decrementando con el tiempo después de remover las termitas de su nido. La decapitación de las termitas paró la actividad inmediatamente. El posible papel de la fijación del nitrógeno asociada con *Nasutitermes* en el ciclo del nitrógeno en Centro Amazonia está discutido.

INTRODUCTION

The regeneration of a tropical rain forest requires a considerable input of nitrogen, part of which may be supplied by biological nitrogen fixation (Nye and Greenland, 1960). Little information is available concerning nitrogen fixation in tropical rain forest. Only since the development of the acetylene reduction technique (Hardy et al., 1968) have such studies been feasible in the tropics. Sylvester-Bradley et al. (1980) found very low rates of acetylene reduction associated with roots of primary forest trees growing on heavy yellow latosol (oxisol) near Manaus, Brazil, even though some of the trees belonged to the family Leguminosae whose members are known for their ability to form nitrogen-fixing root nodules. This lack of nitrogen-fixing activity associated with roots in the rain forest might be due to inhibition of nodulation by the soil, which is acid, low in phosphorus, and has a high aluminium saturation of the cation exchange capacity. An alternative site for nitrogen fixation in the forest might be in the hind guts of termites, which form a large proportion of the soil fauna in Central Amazonia (Fittkau and Klinge, 1973) and which have been shown to possess nitrogen-fixing ability in North America (Benemann, 1973 ; Breznak et al., 1973) and in Australia (French et al., 1976).

Twenty one species of termites were collected from their nests in areas of pasture, primary forest and secondary forest in Central Amazonia. They were tested for nitrogenase activity by the acetylene reduction method. The highest activities (more than $100 \text{ nmol C}_2\text{H}_4 \text{ g dry weight}^{-1} \text{ h}^{-1}$) all occurred in the genus *Nasutitermes* collected in the pasture (Sylvester-Bradley et al. 1978, Figure 1).



A, Amitermes
 B, Grigiotermes
 C, Armitermes
 D, Cornitermes

E, Heterotermes
 F, Labiotermes
 G, Nasutitermes

Fig. 1 - Nanomoles C₂H₄ produced by different genera of termites collected in an area of degraded pasture in Central Amazonia. From Sylvester-Bradley et al., *Acta Amazonica* 8, 621-627, 1978.

Fig. 1 - Nanomoles de C₂H₄ producidas por diferentes géneros de termitas colectadas en un area de pastos degradados de Centro Amazonia.

In the experiments described here termites were sampled on several occasions from the same nests in order to obtain more information about their acetylene reducing activity.

MATERIALS AND METHODS

Preliminary experiments were carried out at a farm (Fazenda NAF-6) on the Manaus- Caracarai road (BV-8). For these experiments the nests were broken open and the termites transferred with a paint brush into 7 ml screw-capped bottles. 10 % acetylene was added to the gas phase and after incubation periods of up to five hours gas samples were withdrawn and analysed for ethylene and acetylene on a gas chromatograph with a flame ionization detector as described by Hardy et al. (1968), see figure 2.

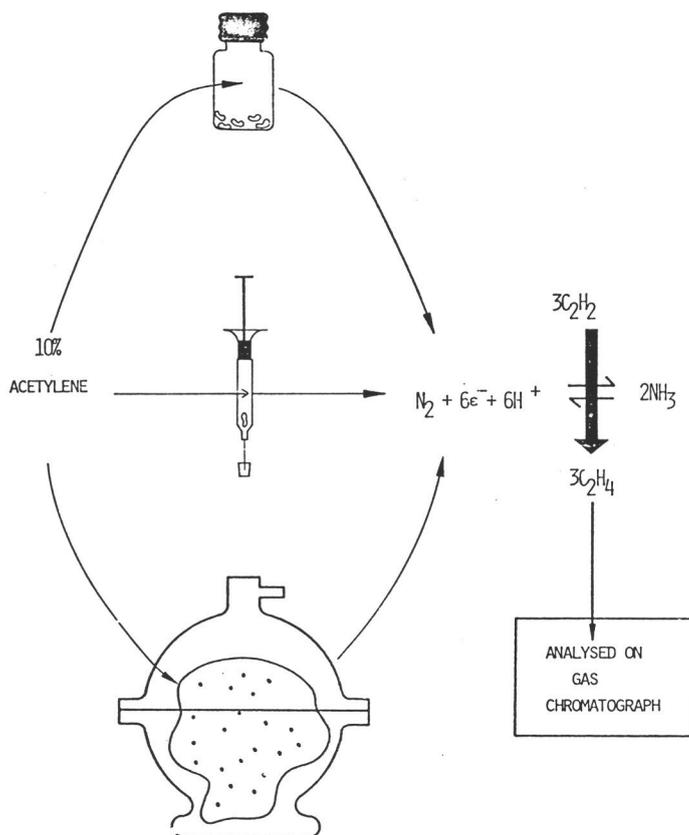


Fig. 2 — Measurement of nitrogen fixation in termites by means of acetylene reduction.

Fig. 2 — Medida de la fijación del nitrógeno en las termitas con el método de la reducción del acetileno.

In further experiments, termites from two nests of *Nasutitermes* spp. on the campus of the Instituto Nacional de Pesquisas da Amazonia (INPA, Manaus) were sampled and tested for acetylene-reducing activity on several occasions by different methods. Large pieces of nest containing termites were placed in desiccators into which 10 % acetylene

was injected, individual termites were placed in the barrels of 1 ml disposable syringes, or approximately fifty termites were placed in 7 ml screw-capped bottles as above (figure 2). Acetylene peak heights were used to correct the ethylene peak heights if the containers leaked.

RESULTS

Acetylene-reducing activities of termites collected on two sampling dates from the same nests at Fazenda NAF-6 did not correlate. Termites from one nest showed high activity on the first sampling date and no activity the following week, whereas other nests showed the opposite response (no activity on the first sampling date and high activity on the second). Three large pieces of a nest of *Nasutitermes* sp. containing termites from INPA collected at the same time and incubated in desiccators showed very different activities, and the activity decreased with time (Figure 3).

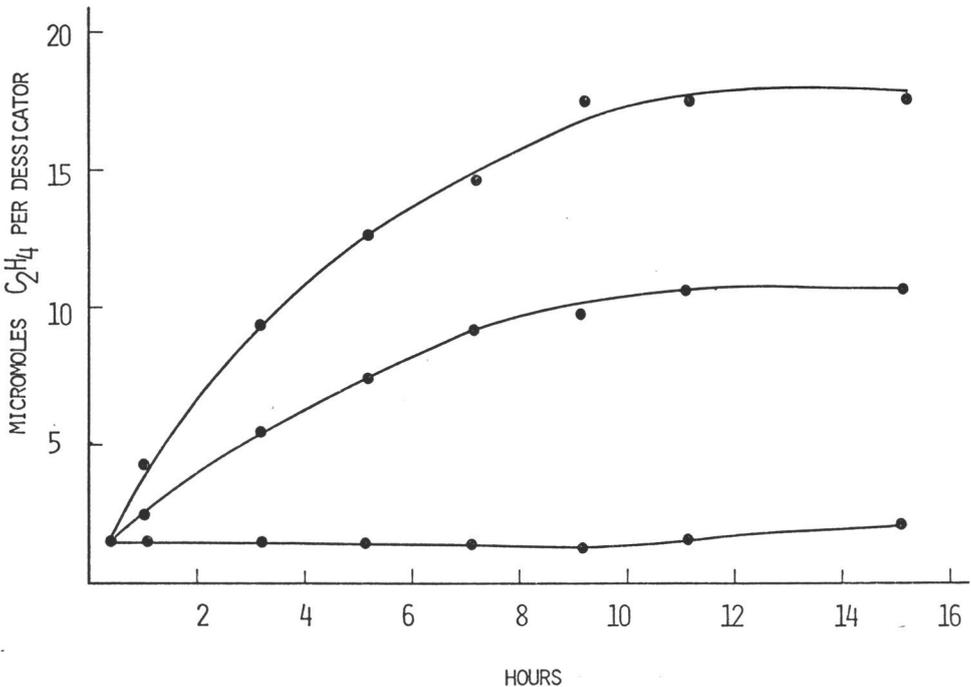


Fig. 3 — Micromoles C_2H_4 produced in desiccators containing *Nasutitermes* sp. within pieces of their nest.

Fig. 3 — Micromolas de C_2H_4 producidas en unos desecadores conteniendo *Nasutitermes* sp. a dentro de pedazos de su nido.

When between thirty and a hundred mixed soldier and worker termites from the same nest of *Nasutitermes* sp. were incubated with acetylene in 7 ml bottles there was no apparent relationship between the number of termites and the amount of acetylene reduced (Figure 4).

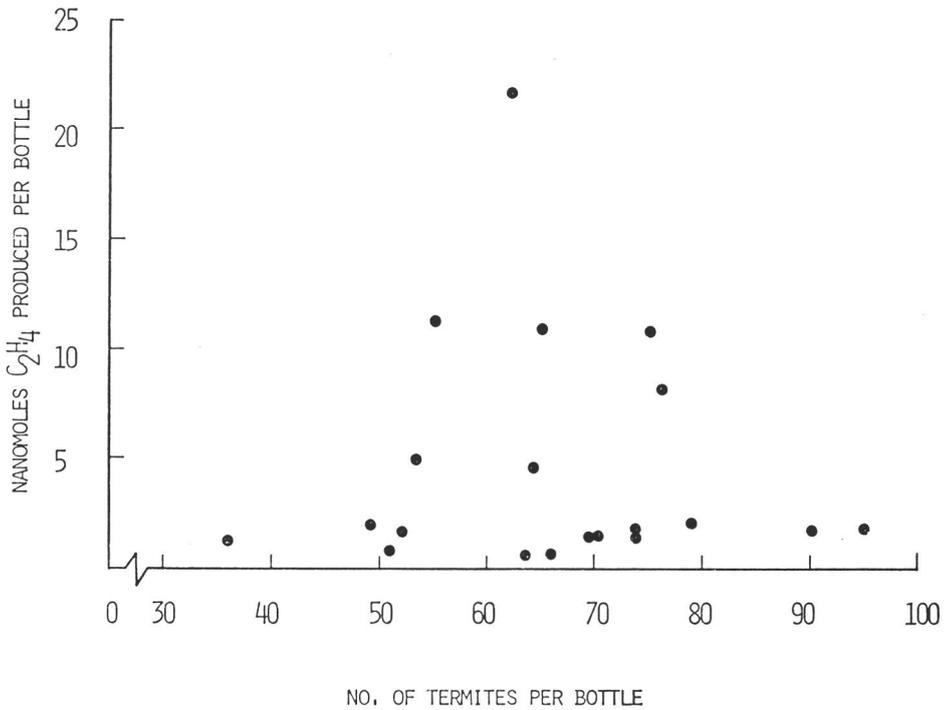


Fig. 4 – Nanomoles C₂H₄ produced in bottles containing between 30 and 100 mixed workers and soldiers of *Nasutitermes* sp.

Fig. 4 – Nanomolas de C₂H₄ producidas en botellas conteniendo entre 30 y 100 obreros y soldados mezclados de *Nasutitermes* sp.

In order to determine whether this lack of consistency in the results was due to differences between individual termites, single worker or soldier termites of *N. macrocephalus* from a nest in INPA were incubated for five hours with acetylene in the barrels of 1 ml disposable syringes. The soldier termites reduced acetylene whereas the workers did not (Table I). By the end of the experiment the soldiers were still alive whereas the workers were dead.

Table I – Nanomoles C_2H_4 produced by individual *N. macrocephalus* termites incubated in 1 ml. syringes with C_2H_2 .

Tabla I – Nanomoles de C_2H_4 producidas por individuos de *N. macrocephalus* incubados en jeringas de 1 ml con C_2H_2 .

Soldiers	Workers
0.08	0.00
0.01	0.00
0.11	0.00
0.07	0.00
0.17	0.00

In a further experiment on mixed worker and soldier termites from the same nest of *N. macrocephalus* some of the termites were deliberately killed by decapitation and their acetelyne-reducing activity compared with that of live termites. The live termites reduced acetelyne whereas the dead ones did not (Figure 5).

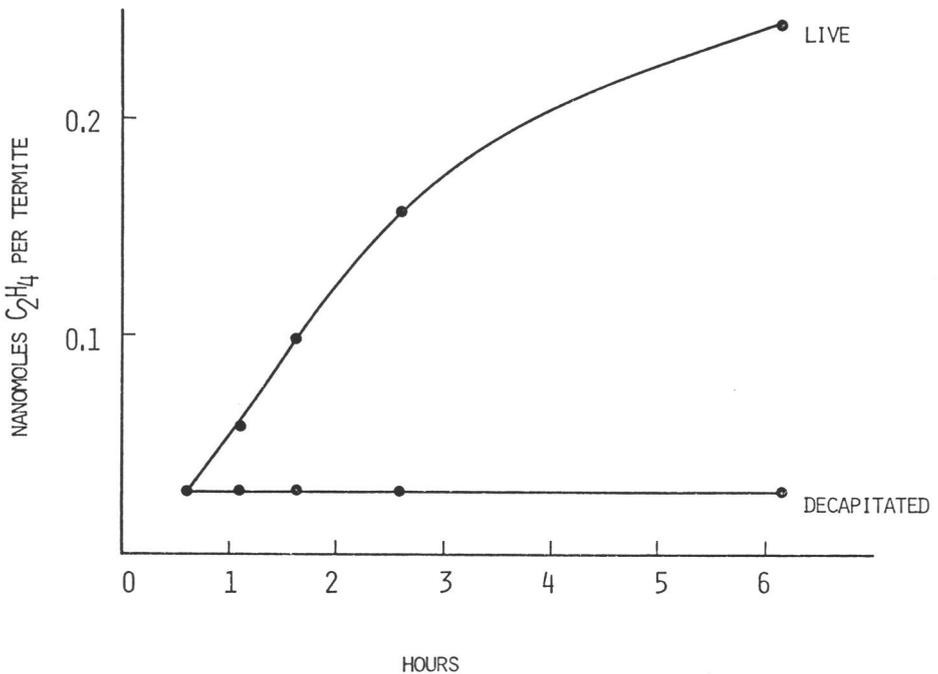


Fig. 5 – Nanomoles C_2H_4 produced by live and dead (decapitated) workers and soldiers of *Nasutitermes macrocephalus* (Mean of 3 replicates).

Fig. 5 – Nanomoles de C_2H_4 producidas por obreros y soldados vivos o muerto (decapitados) de *Nasutitermes macrocephalus* (Promedio de 3 replicados).

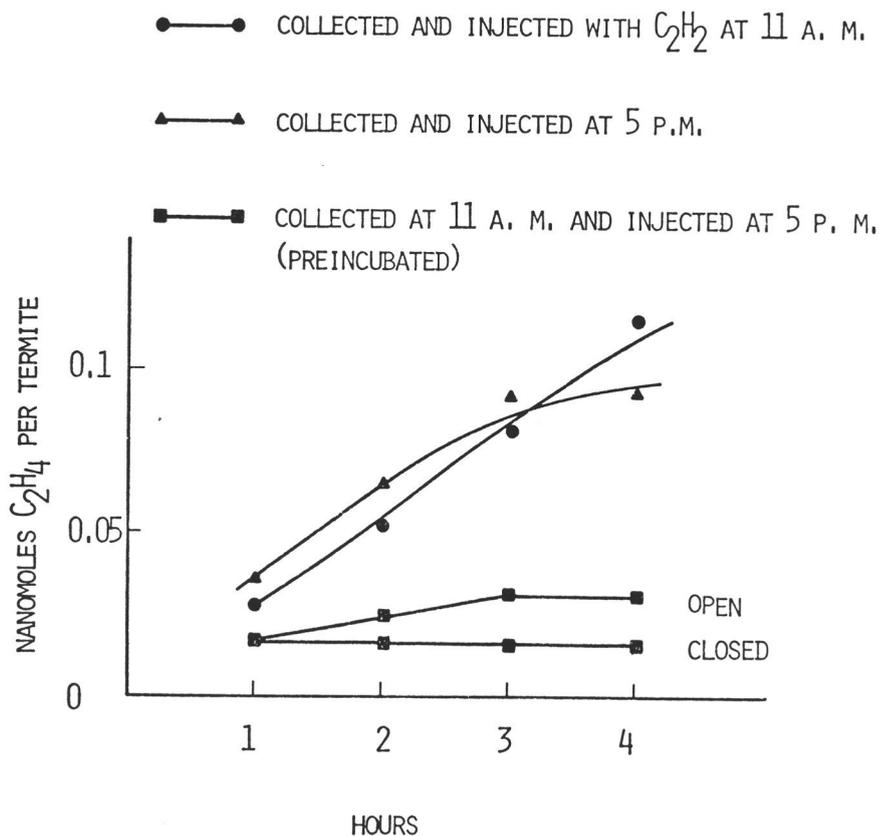


Fig. 6 — Nanomoles C_2H_4 produced by soldier termites of *N. macrocephalus* collected at 11 a. m. or 5 p. m. and exposed to acetylene immediately, or collected at 11 a. and preincubated without acetylene until 5 p. m. Preincubation was carried out either in open or closed bottles.

Fig. 6 — Nanomole de C_2H_4 producidas por soldados de la termita *N. macrocephalus* colectados a las 11 a.m. o 5 p. m. y expuestos al acetileno inmediatamente, o colectados a las 11 a. m. y preincubados sin acetileno hasta las 5 p. m. La preincubación fué llevada a cabo en botellas abiertas o cerradas.

Figure 6 shows that if the acetylene was injected into bottles containing *N. macrocephalus* soldiers immediately after they were collected from their nest, the activity was higher than if acetylene was injected six hours after removal of the termites from the nest. The activity of termites which had been removed from their nest six hours earlier was slightly higher if the bottles containing the termites were kept open during the six hours than if they were closed.

DISCUSSION

The results presented here suggest that dead termites cannot reduce acetylene, even if tested immediately after their death. This is surprising in that the gut microflora of a termite would not be expected to die immediately on the death of its host. However, it implies that the activity of the termite gut microflora depends very closely on the physiological state of its host and that damage or disturbance of the host may inhibit the microflora's activity. The inconsistent results obtained when sampling the same nest more than once may have been due to changes in the behavior and consequently the physiology of the termites in response to disturbance of the nest.

It is possible that the acetylene reduction method underestimates or misrepresents the nitrogen-fixing activity associated with termites. For example Benemann (1973) and Breznak et al. (1973) reported higher activity associated with worker than soldier termites, whereas here we show the opposite result. This might be due to the worker termites tested by us being more fragile than the soldiers, which caused the inhibition of the acetylene-reducing activity associated with them. Sylvester-Bradley et al. (1978) concluded that the low rates of nitrogen fixation associated with *Nasutitermes* spp. in degraded pasture in Central Amazonia could be limiting the rate of decomposition in this ecosystem, and that the total amount of nitrogen fixed would not be sufficient to supply the needs of a regenerating forest calculated by Nye and Greenland (1960).

In order to draw such conclusions it was necessary to assume a ratio of C_2H_2 reduced to fixed N_2 of 3 : 1, which is not necessarily a valid assumption (Hardy et al., 1973). The results presented here indicate that estimates of nitrogen fixation by termites made with the acetylene reduction technique should be interpreted with great care. The rates of acetylene reduction associated with *Nasutitermes* spp. reported by Sylvester-Bradley et al. (1978) indicate that nitrogen fixation occurs in this genus. However, it is not clear whether the rates were under or overestimated or whether the other genera tested are able to fix nitrogen or not.

In view of the important role played by termites in the decomposition process in tropical ecosystems, the limitation of this process by levels of available nitrogen (Aho et al., 1974), and the lack of an appropriate method for estimating the rate of nitrogen fixation associated with termites, it seems too early to conclude that the contribution of the process to the nitrogen economy of the ecosystem is insignificant.

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