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Nitrogen Fixation by Termites in Tropical Forests, Thailand

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Abstract

Nitrogen (N) fixed by termites was evaluated as a N input to decomposition processes in two tropical forests, a dry deciduous forest (DDF) and the neighboring dry evergreen forest (DEF), Thailand. A diverse group of termite species were assayed by acetylene reduction method and only the wood/litter-feeding termites were found to fix N. More intensive samplings of two abundant species, *Microcerotermes crassus* and *Globitermes sulphureus*, were done across several seasons, suggesting N fixation rates of 0.21 and 0.28 kg ha^{-1} y^{-1} by termites in the DDF and DEF, respectively. Also, estimates of asymbiotic N fixation rates were 0.75 and 3.95 kg ha^{-1} y^{-1}. N fixed by termites and by asymbiotic fixers is directly supplied to decomposers breaking down dead plant material and could be a major source of their N. N fixed by termites was 7–22% of that fixed by termites and asymbiotic fixers. Although N fixed by termites is a small input compared to other inputs, this N is likely important for decomposition processes.

Key words: acetylene reduction assay; asymbiotic nitrogen fixation; decomposition process; litter and dead wood; nitrogen fixation by termites; tropical forests.

Introduction

Nitrogen (N) is an element that affects not only primary production (Vitousek and Howarth 1991; Vitousek and others 2002), but also the decomposition of dead plant material (Hunt and others 1988; Vitousek and others 2002). In terrestrial ecosystems, N inputs occur through biological fixation and atmospheric deposition (Jordan 1985; Cleveland and others 1999; Son 2001) and N is lost primarily through leaching and gaseous losses (Vitousek and others 2002; Hall and Matson 2003). Biological N fixation occurs in plant symbiotic, free-living (asymbiotic), and xylophagous arthropod-gut symbiotic forms (Breznak 1975; Cleveland and others 1999; Nardi and others 2002); the latter two forms are expected to directly affect the decomposition process of dead plant material because they are distributed in the surface soil, litter, and dead wood. Despite the many quantitative studies of biological N fixation in the literature, few have considered the contribution of arthropods.

Termites are decomposers widely distributed in temperate, subtropical, and tropical regions. Many termite species utilize N-poor dead plant material and compensate by symbiotically fixing N in their guts (Benemann 1973; Breznak and others 1973; Sylvester-Bradley and others 1978; Bentley 1984; Tayasu and others 1994). Although N fixation rates by termites have been studied by many researchers,

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Table 1. Termite Species Sampled in the Four Sites

Species number and genus species		Feeding group*	Site†	Sampling date‡	No. of nest	Type of workers
1	*Macrotermes gilvus*	WL(F)	Ns	Mar. '03	1	major and minor
2	*Odontotermes* sp.	WL(F)	Ns	Mar. '03	1	major
3	*Termes comis*	S	DEF	Dec. '01	3	-
4	*Dicuspiditermes makhamensis*	S	DEF	Dec. '01	3	-
5	*Schedorhinotermes medioobscurus*	WL	DEF	Feb. '03	1	-
6	*Coptotermes gestroi*	WL	Kas	Oct. '01	1	-
7	*Speculitermes* sp.	WL	Ns	Oct. '01	1	-
8	*Globitermes sulphureus*	WL	DDF and DEF	RsI, Ds	24§	-
9	*Microcerotermes crassus*	WL	DDF and DEF	RsI, RsII, Ds	36§	type-W and -Y
10	*Microcerotermes* spp.	WL	Ns	Mar. '03, Sep. '02	4¶	type-W
11	*Microcerotermes* sp.		Kas	Sep. '01	1	non-specified
12	*Nasutitermes dimorphus*	WL	Kas	Mar. '03	1	-

The numbers of nests used for the experiments are shown with investigated types of workers. Two types of workers are recognized for M. gilvus and O. sp (major and minor workers) and for the genus Microcerotermes (type-W, whitish head type; type-Y, yellowish head type).
**WL, wood/litter-feeders; F, fungus-growers; S, soil-feeders.*
†Ns, Nong Sua; Kas, Kasetsart; DDF, Sakaerat (dry deciduous forest); DEF, Sakaerat (dry evergreen forest).
‡RsI, May–June 2002; RsII, September 2002; Ds, February 2003. §6 nests in each site in each season.
¶3 nests in Mar. '03 and 1 nest in Sep. '02.

only a few studies quantified N fixation by termites at the ecosystem scale. Schaefer and Whitford (1981) showed that the subterranean termite, *Gnathamitermes tubiformans*, fix N at a rate of 0.066 kg ha^{-1} y^{-1} in a desert ecosystem, in New Mexico. Pandey and others (1992) estimated the N amount fixed by *Reticulitermes* spp. in Virginia. These studies, however, did not evaluate N fixation by termites at the ecosystem scale. Because termites are a major decomposer and are diverse and abundant in tropical regions (Wood and Sands 1978; Eggleton and Bignell 1995; Bignell and Eggleton 2000; Eggleton 2000) their contribution to ecosystem N inputs and decomposition processes in tropical ecosystems could be significant.

The aim of the present study was to quantify N fixation by termite assemblages and evaluate their importance by comparison with other biological N fixers in tropical forests. First, possible sources of error in the acetylene reduction assay were evaluated and conditions were chosen to minimize factors that might contribute to unrealistic rates. Second, we measured the N fixation rates of representative species of the termite assemblages in two neighboring forests in Thailand, across several seasons. Asymbiotic nitrogen fixation rates were also measured.

Materials and Methods

Study Sites and Termites

Observations were carried out in two tropical forests, the dry deciduous forest (DDF) and dry evergreen forest (DEF), at Sakaerat Environmental Research Station (14°30′ N, 101°56′ E) at about 500 m above sea level, in the early rainy season (RsI: May–June), late rainy season (RsII: September), and late dry season (Ds: February) from 2002 to 2003. The two dominant species of wood/litter feeding termites, *Microcerotermes crassus* and *Globitermes sulphureus*, were investigated. In addition, as shown in Table 1, various termites were sampled not only in the DDF and DEF, but also at the campus of Kasetsart University in Bangkok and an orchard at Nong Sua near Bangkok (14°11′N, 100°54′E).

Only worker termites were assayed. Workers of *Macrotermes gilvus* were divided into two well-known types, namely major and minor workers, and assayed separately for each type. Because two types of workers (types-W and -Y) can be visually distinguished also for the genus *Microcerotermes*, each type was assayed separately. Type-W is characterized by a whitish head and larger head length (0.79 ± 0.03 mm); type-Y is characterized by a smaller (0.75 ± 0.03 mm) yellowish head. According to Roisin (2000), types-W and -Y are probably female and male, respectively.

Temperature in a Termite Nest

Temperatures in an epigeal nest of *M. crassus* were measured in the DDF using three thermistor SK-L200TH data loggers (Sato Keiryoki MFG, Tokyo, Japan) with one sensor probe each. The data loggers, of which the sensor probes were all capped with plastic tubes to avoid wetting and

attack by termites, were set separately on the nest (ambient) and in the upper part as well as in the center of the nest (near the royal cell). Temperature data were logged at 15-min intervals for two weeks over September and October 2002.

Acetylene Reduction Assay

Laboratory Procedure. Termite nests at the two sites, Nong Sua and Kasetsart University, were removed and immediately returned to the laboratory. Twenty to one hundred workers (about 100–400 mg in weight) were taken from the nests. They were placed in glass screw-capped vials (12.6 ml in volume), in which wet filter paper was laid, with butyl rubber septa. Two to six duplicate samples were usually prepared for each nest. These sampling variations among nests reflect availability and population size of each termite species. About 2.5 ml of the headspace was replaced by the same volume of acetylene gas, resulting in a final atmosphere of 19% acetylene. This manipulation was done within 3 h after collection of the nests in the field. The samples were incubated for 2 h at a temperature between 25 and 30°C. During the incubation period, a 50-µl sample of the headspace of each vial was removed with a gas-tight syringe and injected into a GC—14B gas chromatograph (GC) (SHIMADZU, Kyoto, Japan) equipped with a flame ionization detector and a standard Porapak N column. Ethylene peak heights were calibrated against 202 and 505 ppm ethylene standards to confirm the concentration of acetylene in the headspace. N fixation rates were calculated as ethylene production rates and converted to rates based on N weight using a constant of acetylene reduction to N fixation (=3) (Hardy and others 1973; Bentley 1984).

Preliminary Tests. Because N fixation (acetylene reduction) activity in termites is known to be sensitive to unnatural conditions (Prestwich and others 1980; Lovelock and others 1985; Pandey and others 1992; Waller 2000) such conditions should be minimized. To determine the time over which acetylene production was linear, to test for the effects of temperature on acetylene reduction, and to determine the effect of a time delay between collection and incubation, we conducted the following experiments. Termite samples taken from the nest of *Microcerotermes* sp. collected at Kasetsart in September 2001 (Table 1) were incubated for 8 h, and the headspace gas was injected into a GC at 10-min intervals for the first 3 h and at the end of the incubation period. The ethylene concentration in the vial increased linearly for the first 3 h ($R^2 = 0.99$, $P < 0.001$), whereas the concentrations were low at hour 8. For the nest of *Microcerotermes* sp. collected at Nong Sua in September 2002 (Table 1), six samples for each nest were incubated at 25 and 30°C. For the nests of *Microcerotermes* spp. collected at Nong Sua in March 2003 (Table 1), three samples for each nest were taken and incubated 2, 6 or 24 h after collection of the nests in the field.

Field Procedure. For termites in the DDF and DEF, acetylene reduction assays were performed in the field as follows. Prior to the assays, acetylene gas was stored in glass screw-capped vials with butyl rubber septa (acetylene vial) in the laboratory. About 3 ml of acetylene, drawn from the acetylene vial by injecting water using syringes, was injected into the incubation vial. The air in the incubation vial was homogenized by pumping several times, resulting in a final atmosphere of 19% acetylene. The air in the syringe (3 ml in volume and 19% acetylene) was immediately injected to an empty vial [gas sample vial (1): 12.6 ml in volume] screw-capped with a butyl rubber septum, and then the air in the gas sample vial (1) was homogenized by pumping several times [the rate of dilution = (final air volume in the syringe + the vial volume) / (initial air volume in the syringe)]. After a 2-h incubation in a closed container at a temperature between 25 and 30°C, 3-ml of the head space gas in the incubation vial was sampled by injecting water using syringes. This gas sample was immediately injected into an empty vial [gas sample vial (2)], and the air in the gas sample vial (2) was homogenized by pumping several times. Subsequently, the gas samples in the gas sample vial (1) and (2) were analyzed by GC within three days after the incubations. The concentrations of ethylene in the incubation vials were calculated with the rates of dilution.

Litter, Dead Wood, and Soil

A total of 18 pieces of dead wood were randomly sampled on a 50-m transect in each DDF and DEF in Ds and classified into three categories according to sizes: W1, W2, and W3 with diameters of 1–5, 5–10, and greater than 10 cm, respectively. Six surface soil cores (diameter = 2.2 cm, depth = 6 cm) were also sampled. Six heaps of litter were sampled only in the DEF. These samples were brought to the laboratory and investigated one day after collection. Each plant sample was broken down to small particles, and then 2 g of each plant sample was put in a glass vial (12.6 ml in volume). Each soil core was put in a glass vial (55 ml in volume). N fixation rates of litter, dead wood, and soil were determined

in the laboratory by the same method as for termites. After the incubation, the small plant particles were oven-dried at 80°C for 48 h, and milled using a multi-bead shocker MB501(S) (YASUI KIKAI, Osaka, Japan). Using the milled samples, carbon (C) and N analyses were performed with a CHN analyzer (240C, Perkin Elmer, USA).

Statistical Treatment

Means ± standard deviations are shown for the obtained data. The detection limit of the N fixation rate was usually defined for each nest, because the weights of termites used for the measurements and also final concentrations of ethylene in the incubation vial differed from sample to sample. Accordingly, the detection limit was calculated as: (mean ethylene production rate of samples) − 1.96 × (standard error) > 0. Becasue standard deviations cannot be obtained for nests from which only one or two samples were taken, the detection limits were defined as follows: (mean of two samples or value of one sample) > 0.

Results

N Fixation Rate Methodology

N fixation was not significantly affected by incubation temperature (rates were 317 ± 57.4 and 333 ± 41.6 nmol C_2H_4 g^{-1} h^{-1} at temperatures of 25 and 30°C, respectively). The temperatures of the *M. crassus* nest were 25.0 ± 3.8°C on the nest (ambient), and 25.3 ± 4.7 and 25.8 ± 2.0°C in the upper part and the center of the nest, respectively, indicating that the N fixation rates of *M. crassus* measured between 25 and 30°C likely reflect natural rates.

The N fixation rates 24 h after collection were significantly lower than those 2 and 6 h after collection (Figure 1). However, there was no significant difference in N fixation rate between 2 and 6 hours after collection by two-way ANOVA with Tukey's post hoc test (ANOVA: for ''nest'', $F(2, 26) = 32.71$, $P < 0.01$; for ''time'', $F(2, 26) = 133.33$, $P < 0.01$; for ''nest × time'', $F(4, 26) = 0.97$; Tukey's post hoc test: $P_{2h—24h} < 0.01$, $P_{6h—24h} < 0.01$). Therefore, the undisturbed N fixation rates can be obtained when acetylene reduction assays are performed within 6 h after collection of the nest in the field.

N Fixation Rates Observed

There was large variation in N fixation rates among species, ranging from an undetectable level to the highest level of 1130.8 nmol C_2H_4 g^{-1} h^{-1} for *Microcerotermes* sp. (Figure 2). This variation is usually related to functional groups. Three of the four species of fungus-growers and soil-feeders did not fix N at detectable levels. Only *Odontotermes* sp. fixed N at a low level (3.8 nmol C_2H_4 g^{-1} h^{-1}). In contrast, fixation rates were usually higher than 20 nmol C_2H_4 g^{-1} h^{-1} for all non-fungus-growing wood/litter-feeding species except *Speculitermes* sp. and *Schedorhinotermes medioobscurus*.

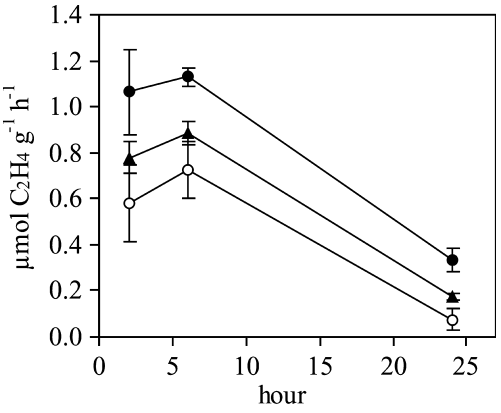

Figure 1. Time courses of N fixation rates after collection in the field. Workers of *Microcerotermes* spp. were picked from nests 2, 6, and 24 h after collection of the nests in the field and incubated in 19% acetylene atmosphere. Different symbols indicate different nests. The *error bars* show standard deviation.

For *M. crassus* and *G. sulphureus*, large intraspecific variation was observed among seasons within a single forest and between the DDF and DEF (Figure 3). N fixation rates were significantly different among nests of *G. sulphureus* in both the DDF and DEF in RsI (for DDF, $F(2, 14) = 27.303$, $P < 0.01$; for DEF, $F(2, 14) = 47.420$, $P < 0.01$, by one-way ANOVA). An inter-caste variation was observed in *M. crassus*, and N fixation rates of type-W were significantly higher than those of type-Y (d.f. = 5, $T = 3.278$–6.587, $P < 0.05$, by paired T-test), except for termites collected in the DEF in RsII and Ds. N fixation rates were significantly higher in the DDF than in DEF for both species (Table 2). A significant seasonal variation was detected from type-Y of *M. crassus* by two-way ANOVA (Table 2), and N fixation rates were higher in RsI than those in RsII and Ds.

N Fixation Rates at the Ecosystem Scale

N fixation rates of termites are calculated at the ecosystem scale using their biomass which was previously obtained in the DDF (A. Yamada and

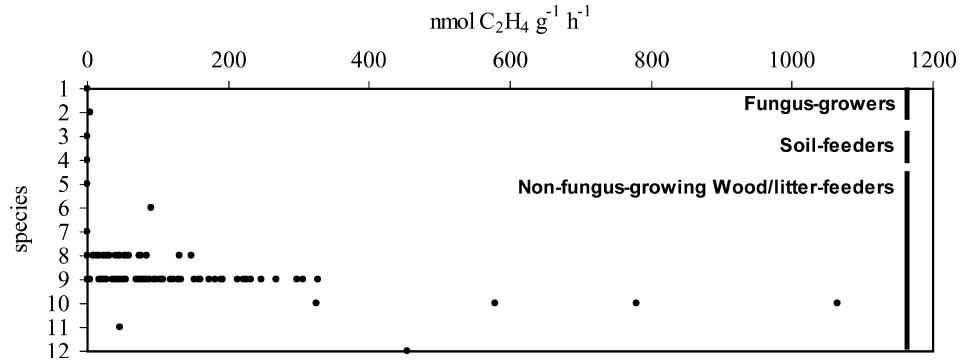

Figure 2. Mean N fixation rates of termites. Species numbers are from Table 1. Each symbol indicates one nest or one caste of one nest.

others, unpublished manuscript) and in the DEF (Yamada and others 2003) and using the N fixation rates from this study. The biomasses of types-W and -Y of *M. crassus* and of *G. sulphureus* are 0.83, 0.83, and 0.23 g m^{-2}, respectively, in the DDF, and 3.01, 3.01, and 1.50 g m^{-2}, respectively, in the DEF, with the assumption that the ratio of the biomass of type-W to -Y is 1:1. Termites in the DDF and in the DEF fixed N at rates of 2.28 and 3.07 mmol C_2H_4 m^{-2} y^{-1}, respectively, assuming that the length of each season is 4 months. For *G. sulphureus*, which was not measured in RsII, rates during that season were assumed equal to rates during RsI. The amounts of N fixed by termites were estimated to be 0.21 and 0.28 kg ha^{-1} y^{-1} in the DDF and DEF, respectively.

N Fixation Rates and C/N Ratios of Litter and Dead Wood

Table 3 shows N fixation rates and C/N ratios for litter and dead wood in the DDF and DEF, using biomass data from A. Yamada and others (unpublished manuscript) and Yamada and others (2003), respectively. The small pieces of dead wood (W1) showed higher N fixation rates in both the DDF (2.39 nmol C_2H_4 m^{-2} y^{-1}) and the DEF (3.56 nmol C_2H_4 m^{-2} y^{-1}) than the larger pieces (W2 and W3), whereas the N fixation rate of litter (Lt) in the DEF (the only site where it was measured) was apparently higher than in all other size classes of wood (4.68 mmol C_2H_4 m^{-2} y^{-1}). The C/N ratios of W1 were usually lower than those of W2 and W3 in each DDF (W1 = 278, W2 and W3 = 356 and 198) and DEF (W1 = 75, W2 and W3 = 117 and 205). N fixation activity was not detected from the soil samples. Using these data, the N fixation rates of all the litter and dead wood at the ecosystems scale were calculated to be 8.1 and 42.3 mmol C_2H_4 m^{-2} y^{-1}, which were converted to 0.75 and 3.95 kg N ha^{-1} y^{-1} in the DDF and DEF, respectively. Average values of the C/N ratio of all the litter and dead wood were also calculated to be 161.0 and 74.9 in the DDF and DEF, using data for the biomass of litter and dead wood in A. Yamada and others (unpublished manuscript) and Yamada and others (2003), respectively.

Discussion

Factors Controlling N Fixation Rates of Termites

The N fixation rates of termites were obtained under conditions in which artificial underestimation was minimized by considering the sensitivity of N fixation activity to unnatural conditions reported in the literature (Prestwich and others 1980; Lovelock and others 1985; Pandey and others 1992; Waller 2000). Thus, it is probable that the rates represent natural ones.

Large intra- and inter-specific variation in N fixation rates of termites as shown in Figure 2 has been reported in several reviews (Breznak 1982; Waller and others 1989; Curtis and Waller 1998; Waller 2000). These reviews, however, report N fixation rates that were measured without considering the delay between collection and incubation. Others have shown that N fixation rates of termites decrease with N concentrations in their food (Breznak and others 1973; Rohrmann and Rossman 1980). In fact, for the smaller size classes of wood, the C/N ratio was higher in the DDF, where the N fixation rates of termites were also higher (Figure 3), than DEF (Table 3). Furthermore, the majority of termites distributed in dead wood have been found in such size classes of wood (unpublished data; Yamada and others 2003); therefore, the intraspecific variation in the N fixation rates of termites between the DDF and DEF (Figure 3) might be explained by wood C/N ratios.

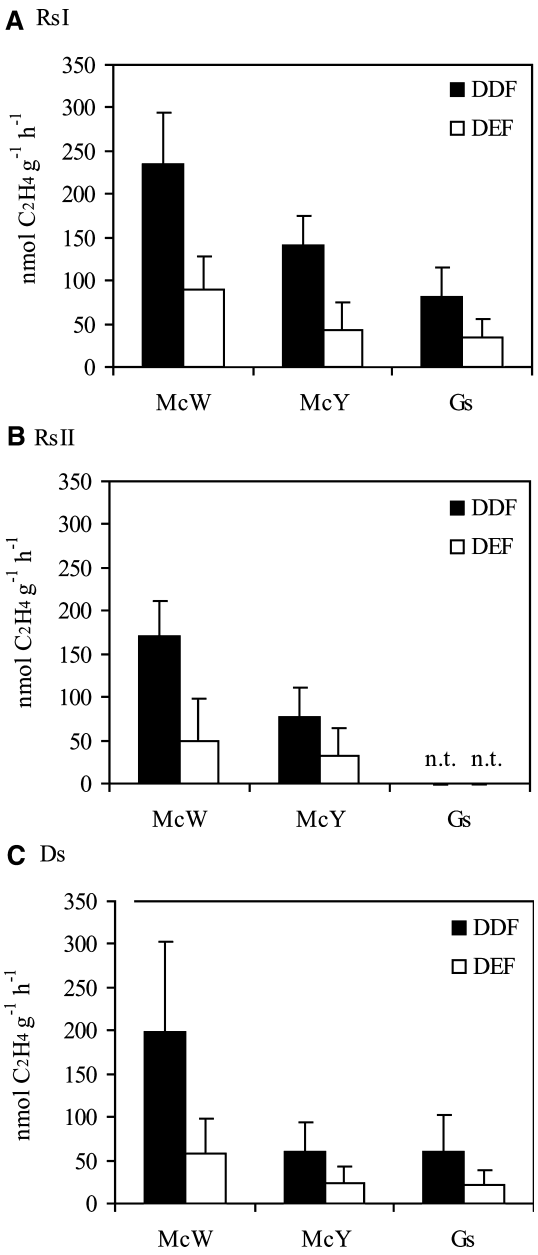

Figure 3. The N fixation rates of workers of *M. crassus*, comprising types-W (McW) and -Y (McY), and of *G. sulphureus* (Gs) in the DDF and DEF. The means and standard deviations of six nests of each species are shown for the three seasons: RsI (May–June) (**A**), RsII (September) (**B**), and Ds (February) (**C**). n.t., not tested.

However, Curtis and Waller (1997) reported no clear correlation between the N fixation rate of *Reticulitermes* sp. and the N content of their food on the field scale. This is not a contradiction if the much higher N fixation rates of *G. sulphureus* and *M. crassus* compared to *Reticulitermes* sp. are more sensitive to and are strongly suppressed by the concentration and available N in foods.

Table 2. ANOVA Tables for N Fixation Rates of Termites

	Source	d.f.	*F*	*P*
(*a*)	Total	35		
	Site (A)	1	44.06	<0.01
	Season (B)	2	2.00	n.s.
	A × B	2	0.28	n.s.
	Error	30		
(*b*)	Total	35		
	Site (A)	1	33.45	<0.01
	Season (B)	2	8.26	<0.01*
	A × B	2	3.83	<0.05
	Error	30		
(*c*)	Total	23		
	Site (A)	1	11.96	<0.01
	Season (B)	1	1.84	n.s.
	A × B	1	0.19	n.s.
	Error	20		

*In both the DDF and DEF, N fixation rates were determined using (a) type-W of M. crassus in RsI (June 2002), RsII (September 2002), and Ds (February 2003), (b) Type-Y of M. crassus in RsI, RsII and Ds, and (c) G. Sulphureus in RsI and Ds. *Tukey's post hoc test: $P < 0.05$ between the DDF in RsI and each of the others; n.s., not significant.*

Importance of N Fixed by Termites

To estimate the amounts of N fixed by termite assemblages in the DDF and DEF, we investigated two species, *M. crassus* and *G. sulphureus*, of non-fungus-growing wood/litter-feeding termites, for which N fixation activity was usually detected in significant levels (Figure 2). Although diverse non-fungus-growing wood/litter-feeders occur in the DEF, the two species are dominant and represented 86 and 96% of the biomass of non-fungus-growing wood/litter-feeders in the DDF and DEF, respectively (A. Yamada and others, unpublished manuscript; Yamada and others 2003).

Here we compare the amounts of N fixed by termites to that fixed by other biological N fixers, including plant symbionts and free-living (asymbiotic) fixers (Figure 4). The amounts of N mediated by plant-symbiotic N fixation have ranged from 0.5 to 60 kg ha^{-1} y^{-1} in tropical evergreen and deciduous forests (Cleveland and others 1999). Asymbiotic N fixation occurs on the surface of litter and dead wood and in the soil; the amounts of N fixed by asymbiotic N fixation were 0.75 and 3.95 kg ha^{-1} y^{-1} in the DDF and DEF, respectively, at the lower end of the range reported by Cleveland and others (1999) for tropical evergreen and deciduous forests (2.5 to 20.0 kg ha^{-1} y^{-1}). Estimates of N in precipitation in tropical forests have ranged from 6.5 to 39.4 kg ha^{-1} y^{-1} (Jordan 1985; Edwards 1982; Strigel and

Table 3. Data on Litter and Dead Wood

Site	Category*	N fixation (nmol C_2H_4 g^{-1} h^{-1}) Ave.	S.d.	C (%) Ave.	S.d.	N (%) Ave.	S.d.	C/N Ave.	S.d.	Biomass‡ (kg m^{-2}) Ave.	S.d.
DDF	Lt	n.t.†		n.t		n.t.		n.t.		0.04	0.03
	W1	2.39	0.77	43.46	7.03	0.28	0.16	278	261	0.25	0.50
	W2	1.66	1.39	45.65	3.58	0.27	0.26	356	257	0.20	0.58
	W3	1.88	4.05	47.78	2.11	0.33	0.25	198	103	0	0
DEF	Lt	4.68	2.06	45.34	3.91	1.33	0.39	37	13	0.61	0.22
	W1	3.56	1.51	47.46	3.45	0.64	0.10	75	14	0.27	0.30
	W2	1.11	0.54	47.17	2.15	0.50	0.27	117	53	0.49	0.41
	W3	0.42	0.64	49.47	1.85	0.33	0.17	205	129	1.13	2.72

N fixation rates and C and N contents were determined using 6 samples for each category.
**Lt, litter; W1, W2 and W3, dead wood with diameters of 1–5, 10, and >10 cm, respectively.*
†n.t., not tested.
‡data from A. Yamada and others (unpublished manuscript) for the DDF, and from Yamada and others (2003) for the DEF.

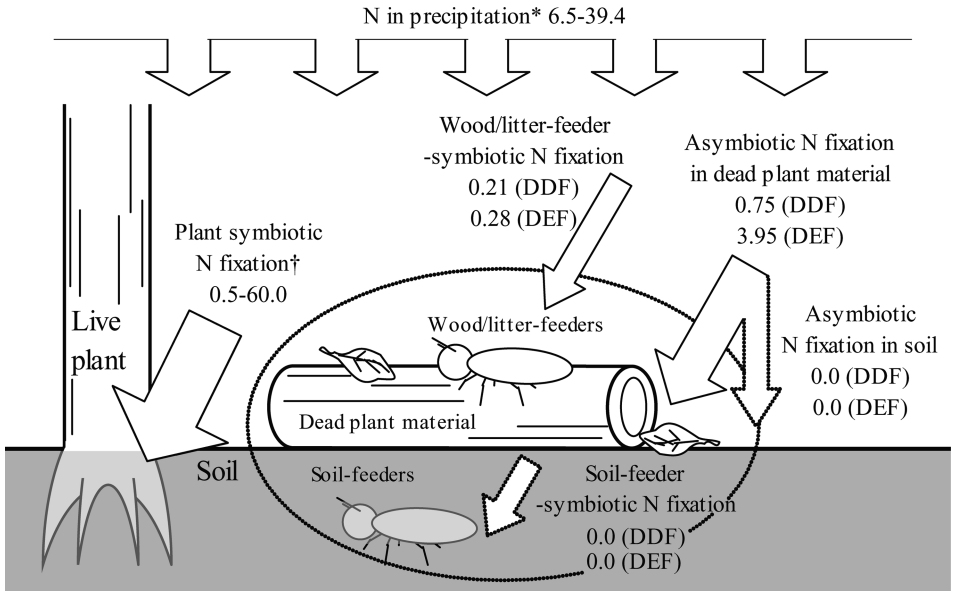

Figure 4. Schematic of N inputs (kg N ha^{-1} y^{-1}) to the DDF and DEF. Only non-fungus-growing wood/litter-feeders of termites fixed N, and asymbiotic N fixation activity was detected from litter and dead wood but not from the soil. * data for tropical forests in Jordan (1985), Edwards (1982), and Strigel and others (1994); † data for tropical evergreen and deciduous forests in Cleveland and others (1999).

others 1994) and are comparable to N inputs from either plant-symbiotic or asymbiotic N fixation.

The amounts of N fixed by termites were 0.21 and 0.28 kg ha^{-1} y^{-1} in the DDF and DEF, respectively. These values are much smaller than those derived through plant-symbiotic and asymbiotic N fixation and precipitation. Although the amount of N fixed by termites is likely a small proportion of the total N input to the DDF and DEF, nevertheless, N fixed by termites likely contributes significantly to the N dynamics of the ecosystems as described below.

Termites are calculated to be responsible for 7–22% of N inputs from fixation to decomposing plant material in the DEF and DDF, respectively (Figure 4). N from precipitation (Jordan 1985; Edwards 1982; Strigel and others 1994) and immobilization by fungal hyphae (Frey and others 2000) may also be potential sources of additional N to decomposing plant material. Taking these po-

tential N sources into consideration, the amounts of N fixed by termites might be of minor importance in decomposition processes. However, from the spatial and temporal point of view, N fixed by termites is expected to contribute significantly to decomposition processes in different ways than the other sources of additional N. Many species of non-fungus-growing wood/litter-feeding termites, for example *M. crassus*, attack and break more or less freshly fallen branches and trunks, and feed on woody material in the center of the wood (Abe 1980) by utilizing fixed N in their guts. In contrast, additional N from asymbiotic fixation, precipitation, and fungus-mediated immobilization is expected be supplied mainly to surfaces of fallen branches and trunks. This is partly supported by the higher fixation rates and lower C/N ratios of smaller size classes of wood (Table 3), which usually have larger surface areas compared to their weights. Here, we suggest that N fixed by termites plays its role in the early decomposition processes and in different parts of the dead wood than the other sources of additional N. N fixed by termites would be excreted as feces out of their bodies. A stable isotope analysis has shown that feces of the termite *Neotermes koshunensis* contain atmospheric N (I. Tayasu, personal communication). It is most likely that at least a portion of feces containing fixed N is put into the center of the wood where the termites are feeding and/or nesting. Therefore, the feces containing N fixed by termites would be an exclusive source of additional N to the centers of freshly dead wood, promoting further decomposition. The present study is the first report on N fixed by termites in tropical forests on the ecosystem scale, in contrast to many excellent ecosystem-scale studies emphasizing the significant roles of C mineralization (Wood and Sands 1978; Martius 1994; Bignell and others 1997; Eggleton and others 1999; Konaté and others 2003).

Various animals prey on termites (Wood and Sands 1978), and thus N fixed by termites is expected to contribute to the N economy of the predators. Becasue termites are one of the most abundant animals in tropical forests (Fittkau and Klinge 1973; Collins 1980) and provide several times larger amounts of their bodies than the biomass to their predators per annum (Wood and Sands 1978), termites could be an important source of N to their predators.

In conclusion, N fixed by termites, though contributing weakly to total N input to the ecosystems, represents a significant part of N input to dead plant material (Figure 4), and importantly affects the decomposition process of dead plant material.

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