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Seasonal patterns of nitrogen fixation in termites

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Summary

1. Termite nitrogenase activity was highest in autumn and spring (≈ 3 μg N_2 fixed termite fresh mass (g)$^{-1}$ day^{-1}) and lowest in winter and summer (≈ 0·8 μg N_2 fixed termite fresh mass (g)$^{-1}$ day^{-1}).
2. The nitrogenase activity of worker termites was significantly higher than all other castes (1·58 ± 0·27 μg N_2 fixed termite fresh mass (g)$^{-1}$ day^{-1}).
3. Worker termites constituted the largest proportion of all the castes throughout the study period (≈ 90%).
4. The localized input of fixed nitrogen by termites may reach 15·3 mg N log^{-1} day^{-1} and 5·6 g N log^{-1} year^{-1}.

Key-words: Biogeochemical cycling, ecology, entomology, forest ecosystems, *Reticulitermes*

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Introduction

Termites may contribute significant amounts of nitrogen to terrestrial ecosystems because their microbial gut flora include nitrogen-fixing bacteria (Breznak *et al*. 1973; Potrikus & Breznak 1977). The newly fixed nitrogen is incorporated into termite tissues and can be distributed throughout the colony by social feeding (trophallaxis) (Bentley 1984; Waller & La Fage 1987). Through this process, termites supplement their nitrogen-poor diet and contribute to the biogeochemical cycling of nitrogen in terrestrial ecosystems (Schaefer & Whitford 1981; Pandey, Waller & Gordon 1992; Slaytor & Chappell 1994; Tayasu *et al*. 1994). However, the amount of nitrogen fixed by termites is unknown. Furthermore, most termites lose their nitrogen-fixing ability within 24 h of laboratory storage and may not regain it until many weeks in the laboratory (Lovelock, O'Brien & Slaytor 1985). Nitrogenase activity in termites is most reliably measured directly after removal of the insects from the field (Pandey *et al*. 1992). Therefore, some reported nitrogen fixation rates are unreliable for calculating ecosystem-level nitrogen inputs by termites.

Termite nitrogen fixation rates vary among termite species (Breznak 1982), and intraspecific variation results from differences in food quality, termite caste, termite size and seasonal factors (Breznak *et al*. 1973; Prestwich, Bentley & Carpenter 1980; Waller, Breitenbeck & La Fage 1989; Curtis & Waller 1995). Nitrogenase activity is higher in soldiers than workers in some termite species and lower than workers in other species (Prestwich *et al*. 1980). Larvae have been reported to fix 300-fold more nitrogen than workers in *Coptotermes formosanus* Shiraki (Breznak 1982). Both nitrogen fixation rates and the proportions of different castes may vary seasonally. Therefore, long-term studies are needed to determine the effects of seasonal factors and colony dynamics on the nitrogen contribution of termites to ecosystems.

In the present study, termite worker nitrogen fixation rates were examined monthly for 35 months in a tidal forest in coastal Virginia, USA. Seasonal caste composition was determined every 2 months throughout the study period, and the nitrogen fixation rates of the different termite castes were measured when they were available. We also estimated termite numbers in logs and calculated the amount of nitrogen contributed per log.

Materials and methods

TERMITES

Wood infested with *Reticulitermes flavipes* (Kollar) and *Reticulitermes virginicus* (Banks) was collected monthly from the headquarters of the Virginia Coast Reserve (VCR) Long-term Ecological Research (LTER) site located in Nassawadox, on the Eastern Shore of Virginia, USA, from June 1993 to April 1996, for the worker assays. Environmental temperature data were obtained from a database maintained by the VCR-LTER (Krovetz & Porter 1993–1996). Termites were also collected from other sites in southeastern Virginia, for worker, soldier, presoldier, nymph, larva and alate assays. *Reticulitermes flavipes* and *R. virginicus* are common to all our study sites. However, termites were not identified to species when no alates were present. Voucher specimens have been deposited at Old Dominion University.

ACETYLENE REDUCTION ASSAY

The acetylene reduction assay was used to determine nitrogenase activity for each termite colony. The assay

is based on the ability of nitrogenase to reduce acetylene (C_2H_2) to ethylene (C_2H_4) at three times the rate it converts dinitrogen (N_2) to ammonia (NH_3) (Hardy, Burns & Holsten 1973; Bentley 1984). The following protocol is adapted from Pandey *et al*. (1992).

Fifty worker termites, removed from their nest material and weighed to the nearest 0·1 mg, were placed in an 8·5-ml glass vial with a rubber sleeve cap septum. One millilitre of air was removed and 1·0 ml of acetylene was added to the vial, resulting in a final atmosphere of ≈ 12% acetylene. Three replicate vials per colony were incubated at 22 ± 2 °C for 30 min. Following incubation, a 200-µl sample of head space was removed with a 0·5-ml Hamilton Gas Tight syringe (Hamilton Company, Reno, NV, USA) and injected into a Varian® 3600 gas chromatograph (Varian Instrument Company, Walnut Creek, CA, USA) equipped with a flame ionization detector and a Porapak® N column (Alltech, Deerfield, IL, USA). Ethylene peaks were analysed using a standard concentration curve with known amounts of ethylene to determine the moles of ethylene produced for each sample. Final nitrogen fixation rates are expressed as dinitrogen fixed (µg) termite fresh mass $(g)^{-1}$ day^{-1}. There were three replicate samples for each colony. Ten colonies per month were sampled for 35 consecutive months. Nitrogenase activity was also measured for the different castes present. All acetylene reduction assays were performed on termites immediately removed from logs within 4 h of taking the logs from the field.

The monthly nitrogen fixation rates of worker termites were analysed using a completely randomized design analysis of variance (ANOVA) to test if there was a difference among the 35 months of the study (α = 0·05) (SAS statistical package, SAS Institute 1990). The nitrogen fixation rates of each termite caste were analysed with a completely randomized design analysis of variance (ANOVA) to test for a difference in nitrogenase activity among the different termite castes over the study period (α = 0·05) (SAS statistical package, SAS Institute 1990) and the Tukey honestly significant difference (HSD) multiple comparison test was used to compare means.

CASTE PROPORTION

Every other month from February 1994 to April 1996, the proportion of different caste members was determined for the same 10 termite colonies that were collected from the VCR and used in the worker nitrogenase activity assays. Termites were removed from their nest material and placed in a 3-mm aperture wire sieve to remove large debris. The volume of the sifted material, including termites, was measured. In three replicate samples, each with a volume containing 250 termites, counts were made for all termites in the following six castes: worker, soldier, presoldier, nymph, larva and alate.

Caste ratio data were transformed (arc-sine square-root) and analysed with a completely randomized design analysis of variance (ANOVA) to test for differences in caste proportion over the study period (α = 0·05) (SAS statistical package, SAS Institute 1990). A completely randomized analysis of variance (ANOVA) was used to test for differences in the abundance of each caste over the season (α = 0·05).

TERMITE ABUNDANCE

Estimates of the number of *R. virginicus* in two logs at the VCR were made by a mark–release–recapture method using the ingestible dye marker Nile blue in March and April 1996. The logs were 7·4 m long × 11 cm in diameter and 2·7 m long × 30 cm in diameter, respectively. Termites were collected from sections of logs in the field, taken to the laboratory, removed from the wood, and fed filter paper impregnated with 0·1% (w/w) of the dye Nile blue for 5 days. A week later, marked termites were counted and released to the original host logs. After 7 days, a 30-cm long section of each log was collected, returned to the laboratory and broken to expose all termites. The broken pieces were then placed into a 3-mm aperture wire sifter to remove the wood particles from the termites. Marked and unmarked individuals were counted and log population estimates were calculated using the Lincoln Index (Petersen method) (Krebs 1989):

$$N = \frac{CM}{R}, \qquad \text{eqn 1}$$

where N = population estimate, M = number of worker termites marked, C = number of worker termites recaptured and R = number of worker termites in the recapture sample that were marked.

Confidence limits (95%) were calculated using the following formula (Krebs 1989):

$$\frac{R}{\mathrm{C}} \pm \left\{ Z_a \left(\frac{\sqrt{(1-f)\left(\frac{R}{\mathrm{C}}\right)\left(1-\frac{R}{C}\right)}}{C-1} \right) + \frac{1}{2}C \right\}, \qquad \text{eqn 2}$$

where f = fraction of total population sampled in the recapture sample ≈ R/M, $^1/_2C$ = correction for continuity and Z_α = standard normal deviate for $(1 - \alpha)$ level of confidence = 1·96 (for 95% confidence limits).

Results

NITROGENASE ACTIVITY

There was a significant difference among monthly termite nitrogen fixation rates for the 35 months of the study (F = 37·02; df = 34, 1015; P < 0·0001) (Fig. 1). These differences were related to seasonal variation in nitrogenase activity with the highest rates occurring in the moderate temperatures of spring and autumn and

the lowest rates occurring in the extreme temperatures of winter and summer. Winter rates were lowest, approximating zero. There was a significant difference in nitrogen fixation rates among the different termite castes (F = 24·78; df = 5, 165; P < 0·0001) (Fig. 2). Worker termites had the highest nitrogenase activity of all termite castes (1·58 ± 0·27 µg N_2 fixed termite fresh mass (g)$^{-1}$ day^{-1}) (Tukey HSD).

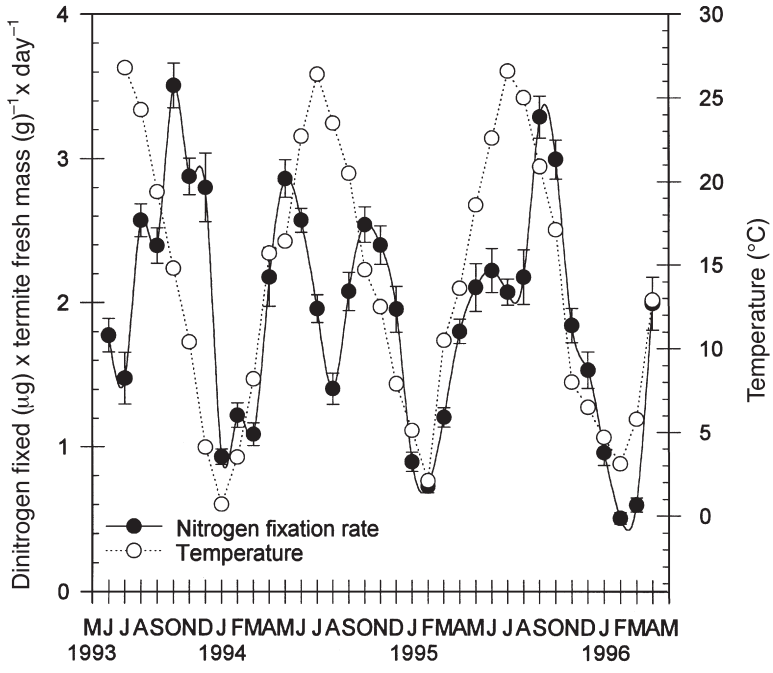

Fig. 1. Termite nitrogen fixation rates (mean ± SE) and monthly mean temperature from June 1993 to April 1996. Each point for dinitrogen fixed is the mean of 30 replicate samples, three each from 10 separate *Reticulitermes* spp. colonies. Replicate samples contained 50 termites.

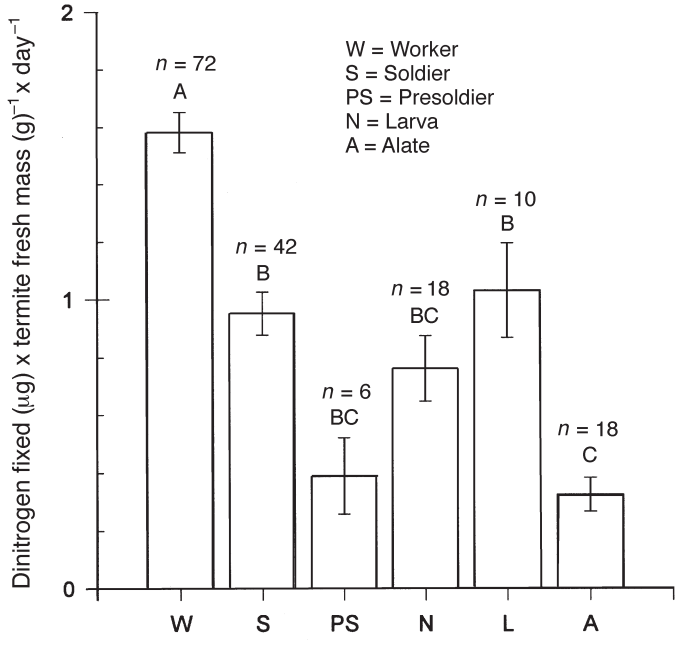

Fig. 2. Nitrogen fixation rates (mean ± SE) of each termite caste: n = replicate group; for workers each group contained 50 termites, for soldier, presoldier, nymph and alate each group contained at least 20 termites, and for larva each group contained at least 200 termites.

CASTE PROPORTION

There was a significant difference in the caste proportion of termites (F = 1282·9; df = 5, 63; P = < 0·001) from 1994 to 1996. Worker termites represented the highest proportion per colony for each collection period (87%), but their caste ratios differed significantly over the bimonthly collections (F = 2·02; df = 13, 126; P = 0·024) (Fig. 3a). Larvae, the second most abundant caste, did not differ significantly in caste ratio over time (F = 1·55; df = 13, 126; P = 0·11) (Fig. 3b) but their proportions tended to be the opposite of worker proportions. There was a significant difference in the relative abundance of soldiers over the study period (F = 2·21; df = 13, 126; P = 0·012) (Fig. 3c). Soldier proportion was lowest in June (0·5%) and highest in April (2·1%). There was a significant difference in the abundance of presoldiers over the study period (F = 8·81; df = 13, 126; $P \leq$ 0·0001) (Fig. 3d). Presoldier proportion was highest in August. There was no significant difference in the abundance of nymphs (F = 0·74; df = 13, 126; P = 0·72) or alates (F = 0·92; df = 13, 126; P = 0·53) over the months of the study (Fig. 3e, f).

TERMITE ABUNDANCE

The population estimates for two *R. virginicus* colonies in logs were 0·49 × 10^6 worker termites for the log measuring 7·4 m long × 11 cm in diameter and 1·78 × 10^6 worker termites for the log measuring 2·7 m long × 30 cm in diameter (Table 1). Based on these log abundance data, *R. virginicus* is capable of contributing 15·3 mg N log^{-1} day^{-1} and 5·6 g N log^{-1} year^{-1}.

Discussion

Nitrogen fixation has been demonstrated in all termite families (Slaytor & Chappell 1994). Given their global distribution, termites may have widespread importance in the biogeochemical cycling of nitrogen in terrestrial ecosystems. Pandey *et al*. (1992) estimated that *Reticulitermes* spp. contribute 125·5–445·3 g N ha^{-1} year^{-1} in a forest ecosystem, while Schaefer & Whitford (1981) estimated *Gnathamitermes tubiformans* (Buckley) contributes 66 g N ha^{-1} year^{-1} in a desert ecosystem. However, those studies did not measure seasonal variation in nitrogen fixation rates.

Termites inhabit fallen logs that occur throughout forest ecosystems. Although nitrogen inputs to ecosystems are usually given per unit area, termite nitrogen contributions may be more ecologically relevant if viewed as a mosaic of nitrogen 'hot-spots' concentrated in termite-infested logs. The uneven distribution

of nitrogen input by termites may contribute to the nutrient-patchiness of forest soils. We estimated the population size of two *R. virginicus* colonies in fallen logs to have 0·49 and $1{\cdot}7\times 10^6$ worker termites. This figure represents an upper limit because mark–release–recapture abundance estimates may be up to 12 times higher than the actual population (Curtis & Waller 1997). Based on these abundance data, termites may contribute up to 15·3 mg N $\log^{-1}$ day^{-1} to logs. This nitrogen may accumulate over a season and be released even during low rates of termite nitrogen fixation. Nitrogen augmentation increases log decomposition by fungi (Swift 1977), so termite nitrogen fixation may speed nutrient release from infested wood.

Workers had the highest rates of nitrogenase activity of any caste. Previous studies have indicated that larvae may fix 300 times as much nitrogen as workers in the rhinotermitid *Coptotermes formosanus* (Breznak 1982). In our study, *Reticulitermes* spp. larvae fixed more nitrogen than other castes except workers. Soldiers of *Nasutitermes* spp. have been reported to have four times the nitrogen fixation rates of workers (Prestwich *et al*. 1980). However, in our study the nitrogen fixation rates of *Reticulitermes* spp. soldiers and presoldiers were significantly less than workers. The nitrogenase activity of nymphs and alates was also significantly less than the workers. Perhaps the inability to supplement their nitrogen-poor diet through nitrogen fixation explains why some newly flown alates feed on the nitrogen-rich cambium layer of logs when they initiate new colonies (Shellman-Reeve 1994).

Table 1. Termite abundance data as calculated by the Lincoln index (Peterson method)

Log	Date	*M*	*C*	*R*	*N* ± SE
7·4 m × 11 cm	March 1996	16 190	8 881	81	1 775 104 ± 397 623
2·7 m × 30 cm	April 1996	15 170	18 361	567	491 245 ± 39 201

M = number of worker termites marked, *C* = number of worker termites recaptured, *R* = number of worker termites in recapture sample that were marked, *N* = estimated number of worker termites in the log and standard error (SE).

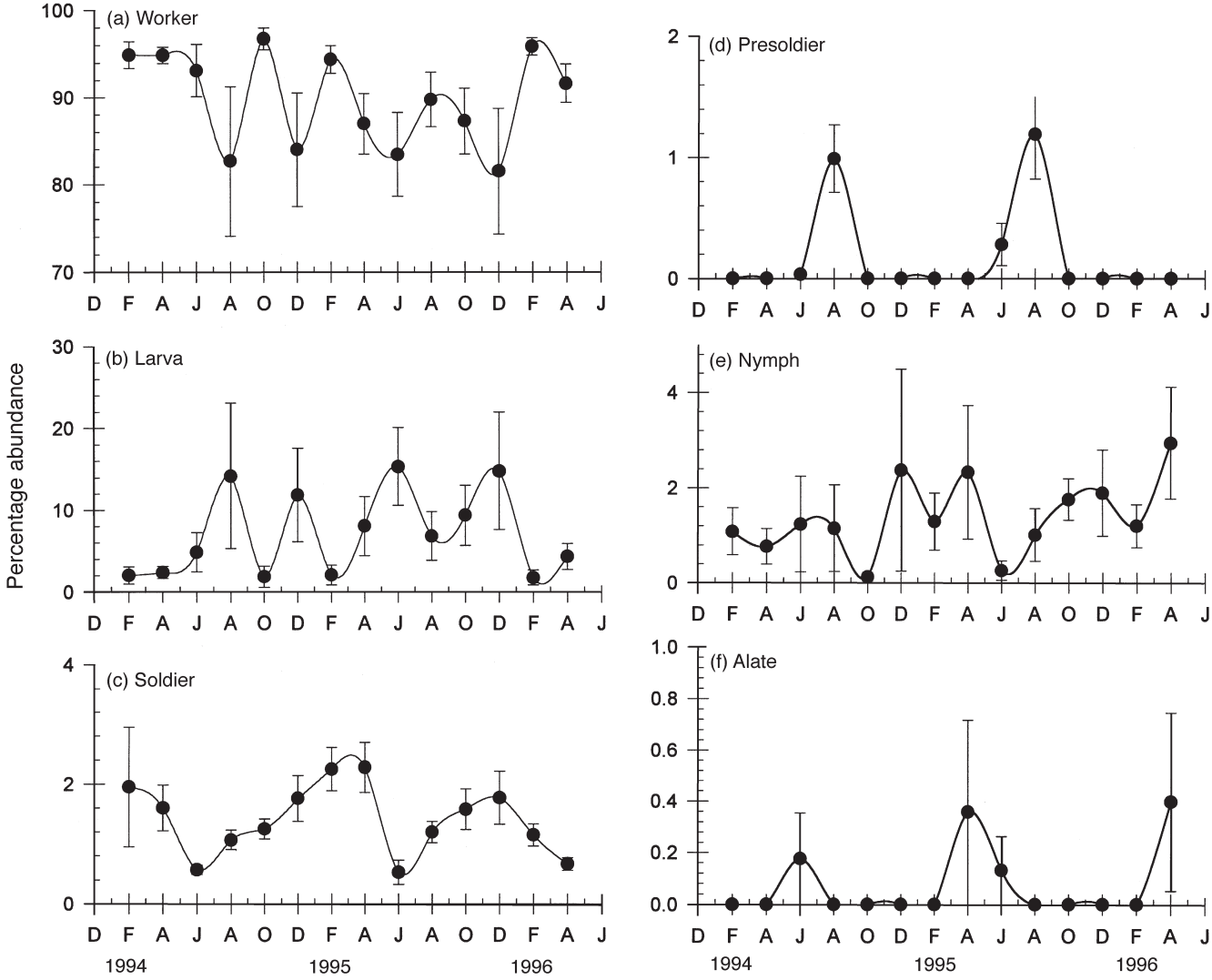

Fig. 3. Percentage abundance (mean ± SE) of (a) worker, (b) larva, (c) soldier, (d) presoldier, (e) nymph and (f) alate from February 1994 to April 1996.

The low rates of nitrogenase activity in non-worker castes coupled with their low numbers in our density samples indicate that their nitrogen contributions to forests are minimal. However, higher numbers of these castes might occur in termite nests deep within the soil. Nitrogenase activity in *Reticulitermes* increases with decreasing oxygen levels (Curtis & Waller 1996), which may occur in subterranean nests. Therefore, nitrogen contributions by termite colonies might be higher than estimated here.

Seasonal fluctuation in the proportion of soldiers in our study was similar to the findings of Howard & Haverty (1981). Soldier proportion varied seasonally with a peak in spring at ≈ 2%. The seasonal low in soldier proportion (≈ 1%) in summer was immediately followed by a peak abundance in presoldiers at ≈ 1%. The decrease in soldier proportion occurred at the same time as alate reproductive proportion peaked, perhaps because soldiers were lost while protecting alates.

In conclusion, we showed that termite nitrogen fixation rates varied seasonally and rates were highest in the moderate temperatures of autumn and spring. The seasonal lows in termite nitrogen fixation rates occurred in summer and winter when temperatures are at extremes. Because the nitrogen fixation rates and abundance were low for the non-worker castes, their nitrogen contribution to forests is likely to be minimal. The pattern in which termites deposit nitrogen to forests may influence the nutrient-patchiness of forest soils and may therefore affect the distribution of other species in that habitat.

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