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ORIGINAL ARTICLE

Estimation of food composition of *Hodotermes mossambicus* (Isoptera: Hodotermitidae) based on observations and stable carbon isotope ratios

Craig T. Symes[1] and Stephan Woodborne[1,2]

[1] *School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Wits,* [2] *Natural Resources and the Environment, CSIR, Pretoria, South Africa*

Abstract The diet of the harvester termite *Hodotermes mossambicus* was investigated at two sites with distinct dietary components: C_4 grasses ($\delta^{13}C$ isotope values, −13.8‰ to −14.0‰) and C_3 plants ($\delta^{13}C$ isotope values, −25.6‰ to −27.1‰). By comparing observations of food items carried into the colony by the termites and carbon isotope ratios of whole termites (that determined assimilated carbon), the relative proportion of the C_3 and C_4 plant food components of the termite diet was estimated. There was agreement between the observational data and stable carbon isotopic data, with grass representing approximately 93% of the diet of *H. mossambicus* at two study sites (urban and rural) on the South African highveld. However, when correcting for mass of food items, that is, C_3 and C_4, carried by termites, the proportion of grass (C_4) in the diet may be underestimated.

Key words carbon assimilation, colony, harvester termite, Suikerbosrand

Introduction

Termites are important soil organisms that play a significant role in soil-forming processes (LaFage & Nutting, 1978; Hewitt *et al.*, 1990). By decomposing and recycling plant material, they contribute to soil fertility and the global cycling of carbon, nitrogen and other elements (LaFage & Nutting, 1978; Hewitt *et al.*, 1990; Tayasu, 1998). The harvester termite *Hodotermes mossambicus* (Hagen) (Isoptera: Hodotermitidae) has a widespread distribution from the semi-arid areas of southern South Africa, through central and east Africa to Ethiopia, wherever the average annual rainfall is < 750 mm (Coaton & Sheasby, 1972, 1975). Its diet consists primarily of ripe and/or frost- or drought-killed grass but it also consumes tree and shrub material (Nel & Hewitt, 1969; Hewitt *et al.*, 1990). Colonies consume considerable quantities of plant material, particularly during the dry winter months, and are capable of removing a large proportion of standing crop, as well as litter (Nel & Hewitt, 1969; Duncan & Hewitt, 1989; Duncan *et al.*, 1990; Hewitt *et al.*, 1990; Duncan, 1997). Most studies of *H. mossambicus* have focused on the major dietary item of grass (C_4), and less so on the importance and/or contribution of detrital shrub (C_3) material (Duncan & Hewitt, 1989; Hewitt *et al.*, 1990). In addition, no studies that we know of have considered stable isotope analysis to investigate diet.

Diet determination of termites has traditionally been from observations, but more recently dietary studies have used stable isotopes (e.g., Spain & Reddell, 1996; Tayasu *et al.*, 2002; Bourguignon *et al.*, 2009). Because the stable isotope ratio of a consumer's tissue reflects that of the food, stable carbon isotopes offer the opportunity to quantify assimilated carbon from C_3 and C_4 sources in termite colonies, rather than ingested or collected material alone (Peterson & Fry, 1987; Tayasu *et al.*, 1997; Kelly, 2000; Bourguignon *et al.*, 2009). C_3 and C_4 plants fix carbon isotopes (^{12}C and ^{13}C) in different ratios as determined

Correspondence: Craig T. Symes, School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits 2050, South Africa. Tel: +27 11 717 6423; fax: +27 11 717 6494; email: craig.symes@wits.ac.za

by the photosynthetic pathway. In C_3 plants, because ^{13}C is discriminated against more strongly than in C_4 plants, $^{13}C/^{12}C$ ratios are more depleted relative to C_4 plants (Park & Epstein, 1960, 1961; Smith & Epstein, 1971). Typical $\delta^{13}C$ values of *c.* −26.5‰ (−37‰ to −24‰) and *c.* −12.5 ‰ (−16‰ to −9‰) are recorded for C_3 and C_4 plants respectively (Vogel *et al.*, 1978; Ehlringer & Osmond, 1989; Ehlringer, 1991; Dawson *et al.*, 2002). This bimodal pattern of $^{13}C/^{12}C$ ratios of C_3 and C_4 plants can therefore be used as a tool to reconstruct the proportion of assimilated carbon from different plant types in the diet of *H. mossambicus*. The dietary reconstruction relies on a linear mixing model, with the proportions of two food sources in an animal's diet calculated as follows:

$$\delta X_{\text{tissue}} = p\delta X_A + (1 - p)\delta X_B + \Delta,$$

where δX_{tissue} is the isotope ratio in the animal's tissue, δX_A and δX_B are the isotope ratios of the respective food sources and p is the proportion of food A in the diet. The fractionation factor, which can be negative or positive, represents the change in isotopic signature from the diet to tissue, and is represented by Δ (Hoefs, 1980; McKechnie, 2004).

We hypothesized that carbon isotope analysis would support findings of dietary proportions determined from observations of feeding *H. mossambicus* during the same period. In turn it was predicted that these dietary proportions would be similar to dietary proportions when masses of different plant types (i.e., C_3 or C_4) carried by termites into the colony were accounted for.

Methods

The study was conducted at two different sites, *c.* 80 km apart, in Gauteng Province, South Africa. At the Council for Scientific and Industrial Research site (CSIR; 25°45′S 28°17′E), Pretoria, a termite colony above the subterranean radio-C^{14} dating facility of the Quaternary Dating Research Unit, was studied. Two locations at this site within 20 m of each other were sampled; one in open grassland (C_4) and one beneath an exotic tree (*Tipuana tipu*) (C_3). At the Suikerbosrand Nature Reserve site (SNR; 26°32′S 28°10′E), data collection occurred at three locations in open bushveld habitat, comprising a mixture of C_4 grasses and C_3 trees and bushes, in the west of the reserve; all locations were approximately 20 m apart. Both sites, representing an urban (CSIR) and rural environment (SNR), were sampled during May–June 2006, when termites were actively harvesting dry plant material during the day (Mitchell *et al.*, 1993).

Observation data

Foraging termites (minor workers) on the ground surface were counted (scan sampling) to determine the proportion of plant material transported into the nest/colony, and hence the proportion of C_4 grass and C_3 leaf components in the diet. The number of termites carrying grass items as a proportion of all termites carrying food items was used to calculate the proportion of C_4 in the diet of termites. Termites not carrying loads were excluded from any analyses. Sampling occurred for 10–30 min sessions during the mid-morning and early-afternoon when termite foraging activity was high. At two SNR locations the mean weight of dried grass and leaf samples were calculated from 10 grass samples and five leaf samples collected from termites. Combined loads for each vegetation type, at each location, were weighed on a Mettler Toledo AG64 scale (Greifensee, Switzerland) to the nearest 1 μg and divided by the number of termites from which samples were collected, to determine the mean mass of load for a single termite. Dietary proportions of each vegetation type that accounted for differences in masses of C_3 and C_4 dietary components were then calculated. We corrected for mass by multiplying the average mass of different dietary types (C_3 leaf or C_4 grass) at each location by the number of food items carried at each location (C_3 leaf or C_4 grass), and then calculated the proportion of dietary components (C_3 leaf or C_4 grass) as a fraction of the total mass carried by observed termites.

Isotope analysis

Ten termites, 30 grass samples and 10 leaf samples taken from individual termites, were collected from each location at the CSIR. At two locations at SNR, 10 and five termites with their grass and leaf loads respectively were collected. These plant samples represented the C_3 and C_4 end-points for determining dietary proportions of termites.

Because the isotopic fractionation from diet-tissue for *H. mossambicus* is not accurately known, an isotopic fractionation factor of 0.6‰ was used (Spain & Reddell, 1996). To account for any variation around this value, we also used a range of likely fractionation factors (0.5‰–1.0‰) to demonstrate the possible proportions of carbon assimilated from C_3 plants and C_4 grass (Table 2).

Vegetation samples were washed in a weak acid solution (2% HCl) to remove external contaminants, and termites rinsed briefly in 75% ethanol and oven dried at ≈ 80°C. Whole termite samples, including gut contents which were assumed to represent a small fraction of the overall mass of each termite, were then ground and weighed into tin cups (pre-cleansed in toluene) for combustion at 1 000°C to CO_2 in a Flash Elemental Analyzer (11 12 Series, Thermo™, Thermo Fisher Scientific, Bremen, Germany). The elemental analyzer was coupled to a Delta V Plus continuous-flow isotope ratio mass spectrometer (Thermo Finnigan, Delta V Plus, Bremen, Germany) by means of a Conflo III device (Thermo™). One laboratory standard (dried chicken blood; mean $\delta^{13}C \pm SD = -17.87 \pm 0.15$‰; $n = 331$) was measured for every six unknowns. Isotope ratios are expressed in δ notation in parts per thousand (‰) relative to the Vienna Pee Dee Belemnite standard. The laboratory standard was calibrated against C652 ANU sucrose, 1577b (National Institute of Standards and Technology, USA [NIST]) bovine liver and 1547 peach leaves (NIST).

Results

Observation data

Termite activity did not appear to change during the observation periods (C.T. Symes pers. obs.). Nine hundred and eighty-five termites were counted moving toward a mound entrance carrying loads of plant material (Table 1). An additional 56 observations were of termites with no load and were rejected in further analyses. For those termites that were observed carrying plant material the color and texture of the material clearly indicated its origin as grass (green to white in color with a distinctive shape and texture) or detrital leaf material (typically brown in color and irregularly shaped, with a distinct leafy texture). At the location beneath the tree at the CSIR we cannot be sure of the plant species identification for loads carried by termites. We do suspect, given the proportion of *T. tipu* leaves collected beneath the tree, that termites did collect leaf material of this exotic species. Overall observation indicated that grass (C_4) food items comprised 93.8% of the diet of termites. Proportions of grass in the diet were similar at both sites (CSIR 93.6%, SNR 93.9%; *t*-test dependent samples, $t = -0.40$, $P = 0.76$; Table 1). There was slight variation in the proportions of material between locations at the CSIR and at SNR, which is attributed to small sample sizes and chance (Table 1).

Samples were only weighed at SNR (Table 1). The mass of C_4 food items was generally lighter (66.4 and 48.6 mg, locations 1 and 2, respectively) than the C_3 food items (67.2 and 75.9 mg, locations 1 and 2, respectively) although not significantly so (*t*-test independent samples, $t = -1.42$, $P = 0.29$). The dietary proportions, accounting for differences in masses of food items at each location, are given in Table 1.

Isotope analysis

Isotope $\delta^{13}C$ values for grass and leaves carried by termites, and for whole termites, are given for both study sites in Table 2. At the CSIR the proportion of grass in the diets of termites beneath the tree was slightly less than the grass site. At two locations at SNR termites carrying grass had slightly more positive $\delta^{13}C$ values ($\delta^{13}C = -15.1$‰ and -15.3‰) than leaf-carrying termites ($\delta^{13}C = -15.6$‰ at both sites), although not significantly so (*t*-test independent samples, $t = 4.00$, $P = 0.06$). The overall proportion of grass in the diet between the two sites (CSIR and SNR) was similar (*t*-test dependent samples, $t = -0.33$, $P = 0.80$; Table 2).

Table 1 Percentage of C_3 (tree and shrub leaves) and C_4 (grass) dietary components of *Hodotermes mossambicus* termites determined from observations at two sites in Gauteng Province, South Africa (Council for Scientific Research, CSIR and Suikerbosrand Nature Reserve, SNR).

Site	Location	Observations		Mass corrected	
		% C_3	% C_4	% C_3	% C_4
CSIR	Tree location	8.8 (16)	91.2 (166)	–	–
	Grass location	4.3 (9)	95.7 (199)	–	–
	Mean	6.4 (25)	93.6 (365)	–	–
SNR	Location 1	9.1 (18)	90.9 (179)	10.8 (5)	89.2 (10)
	Location 2	3.7 (7)	96.3 (180)	16.9 (5)	83.1 (10)
	Location 3	5.5 (11)	94.5 (200)	–	–
	Mean	6.1 (36)	93.9 (559)	14.4	85.6

Mass corrected dietary proportions are calculated by accounting for differences in mean mass of C_3 and C_4 food items at two SNR sites. Sample size given in parentheses.

Table 2 δC^{13} (‰Vienna Pee Dee Belemnite standard) values of food items (C_3 grass and C_4 tree and shrub leaves collected from *Hodotermes mossambicus* termites) and whole termites at two sites in Gauteng Province, South Africa (Council for Scientific Research, CSIR and Suikerbosrand Nature Reserve, SNR).

Site	Location	C_3	C_4	Termites	% C_4 (grass) in diet
CSIR	Tree location	−25.6 (10)	−13.9 (30)	−15.7 (10)	90.0 (89.2–93.4)
	Grass location	−26.9 (10)	−14.0 (30)	−15.3 (10)	94.3 (93.6–97.4)
	Mean	−26.2	−13.9	−15.5	92.2 (91.4–95.4)
SNR	Location 1	−27.1 (5)	−13.8 (10)	−15.3 (15)	93.0 (92.3–96.0)
	Location 2	−26.7 (5)	−14.0 (10)	−15.5 (15)	92.8 (92.0–95.9)
	Mean	−26.9	−13.9	−15.4	92.9 (92.1–96.0)

Percentage C_4 in diet is calculated using a diet-tissue isotope fractionation factor of 0.6‰ (Spain & Reddell, 1996). The possible range of the percentage C_4 contribution is calculated using a diet-tissue isotope fractionation factor of 0.5‰–1.0‰, and indicated in parentheses. Other values in parentheses indicate sample sizes.

When comparing the proportion of grass in the diets of termites using the two techniques, that is, observations (93.8%) and stable isotopes (92.6%), no significant difference was recognized (t-test dependent samples, $t = 0.84$, $P = 0.46$).

Discussion

Observations

A widely used method of studying feeding habits in termites is by assessing the choice of baits presented to termites in varying situations (Wood, 1978). At the CSIR, slightly more termites at the site beneath the tree carried leaves, possibly because there was more leaf material at this location compared to the location situated in the open grass area. However, we cannot be sure that this leaf material was from the tree shading the location. Also, these slight differences between locations at each site may simply reflect a greater availability of leaf material or variability in sampling. However, on average our observation results at two different sites are relatively consistent in supporting previous studies that indicate that the diet of *H. mossambicus* is made up of predominantly grass (Nel & Hewitt, 1969; Hewitt *et al.*, 1990).

Hodotermes mossambicus food items have been shown to vary significantly in mass and size with termites recorded carrying a wide range of different-sized food items (0.1–152.8 mg; Duncan & Hewitt, 1989). It is therefore not surprising that the measured mass of dried forage items in our study fell within this range (Duncan & Hewitt, 1989). The proportion of each component in the diet of termites was expected to be similar to observations when differences in the mass of different food types (i.e., C_3 and C_4) were accounted for, but this was not the case. When the masses of grass and leaf loads were accounted for (at two locations at SNR) the results differed by $\approx 5.5\%$ from the values obtained by counting the proportion of individuals carrying different food items, and by isotope analysis (to determine this difference we only considered the two locations at SNR where samples were weighed). Variation in the mass of food items was not accounted for in our study and this may explain the anomaly in our results. The variation in mean mass, and possible inaccuracies due to small sample sizes, may have resulted in a mismatch between results by mass correction and observations. Other reasons, in addition to those mentioned above, may also explain this difference. For example, *H. mossambicus* may digest and assimilate diets with different C : N ratios (i.e., C_4 grass and C_3 leaf) quite differently (Tayasu *et al.*, 2002). It is therefore suggested that accounting for mass is a less accurate method for determining dietary proportions, and that observations that agree with stable isotope analysis (that measure assimilated material, see below), are a simple and efficient way to determine C_3 and C_4 dietary proportions of termites for recent time periods, particularly if accurate diet–tissue fractionation factors can be determined.

Isotope analysis

By analyzing the isotopic composition of termites and their food base the proportion of carbon from C_4 grass and C_3 leaf assimilated in the diet of *H. mossambicus* can be calculated. Although the diet–tissue fractionation factor for *H. mossambicus* is not known, a value similar to that found in three Australian termite species, where the diet was confidently known, could be used (Spain & Reddell, 1996). Assimilated carbon for C_4 grass (92.9%) was thus calculated on average and was remarkably similar to that of observations (93.8%; Tables 1 and 2). Although the application of an unknown diet–tissue fractionation factor may appear inaccurate, it is well within the range of expected diet–tissue fractionation values expected for *H. mossambicus*. However, a small range of diet–tissue fractionation values may not significantly affect the final interpretation of dietary proportions. If, for example, a fractionation factor range of 0.5‰ (i.e., 0.5‰–1.0‰) is used, the dietary contributions as determined from stable isotopes vary by approximately 3.9% (see Table 2).

Foraging behavior studies of *H. mossambicus* have shown a division of labor among worker castes, with foraging in the feeding area being performed by major workers that mostly cut plant material for transportation and minor workers that mostly transport material (Duncan & Hewitt, 1989; Duncan, 1997). In studies of Australian termites, slight differences ($< 1‰$) in the isotope values between workers and soldiers have been identified (Spain & Reddell, 1996) and in *Drepanotermes rubriceps* (Froggatt) (Termitidae), an Australian termite, neither $\delta^{13}C$ nor $\delta^{15}N$ values differed between minor and major workers (Tayasu *et al.*, 2002). In this study we selected minor workers so our results are insufficient to interpret the use of resources by different castes. We therefore cannot explain the reasons for small differences, albeit insignificant, in $\delta^{13}C$ values of termites carrying leaf and grass material because termites are unlikely to eat the load they carry. In *H. mossambicus* colonies it has been shown that the large larvae (sixth instar) digest and distribute food within the colony (Nel *et al.*, 1969). The dietary proportion calculated from observations is thus likely to represent that of the colony because the dietary items digested by the sixth instars are distributed throughout the colony. It is unlikely that natural selection would select for workers that collected plant material unacceptable to the larvae (Nel *et al.*, 1969). A better understanding of the distribution of food within the colony would be required to predict any trophic niche difference among castes.

Observational and isotopic evidence of diet do not account for seasonal or daily variation in harvesting patterns that may be determined by food availability. Carbon isotope values of termites represent assimilation of carbon from recent resource utilization, whereas observations give an indication of the proportion of C_3 and C_4 in the diet over a shorter time period (i.e., that of the observation period). Tissue turnover of the termites are likely relatively rapid, so stable isotope data reflect recent resource utilization (Gratton & Forbes, 2006), representing days to weeks prior to observations. Because the dietary proportions determined from observations were remarkably similar to that determined from isotope analysis, it is suggested that *H. mossambicus* termites have a consistent intake of C_3 and C_4 into the colony over time, and that resources in the colony are relatively evenly distributed.

Concluding remarks

Because stable carbon isotope ratios are an accurate average representation of assimilated carbon in the diet, they are a useful tool in dietary reconstructions. In this study observations agreed favorably with isotope data and can therefore be viewed as sufficiently reliable in reconstructing *H. mossambicus* colony diets. Because stable isotopes are able to provide insight into animal diets, further studies may provide additional information on African termite biology. In termite species that use covered foraging tunnels, for example, *Odontotermes badius*, where direct observations are not possible, stable isotope analyses may provide detailed dietary information. Future research may involve studies investigating seasonal and annual changes in dietary proportions, the use of capital and income resources in termite colonies that relate to seasonal cycles, and the identification of caste structures in termite and other colonial species (Duncan, 1997; Bourguignon *et al.*, 2009).

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