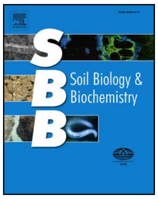
Soil Biology and Biochemistry 117 (2018) 125–134

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio



Spatio-temporal variations of carbon and nitrogen in biogenic structures of two fungus-growing termites (*M. annandalei* and *O. yunnanensis*) in the Xishuangbanna region

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ARTICLE INFO

Keywords:
Macrotermes annandalei
Odontotermes yunnanensis
Physical and chemical properties
Termite mounds
Chronological development
Fungus-growing termites

ABSTRACT

Fungus-growing termites are abundant and play an important role in soil nutrient dynamics in the tropics due to their activities and unique effects on soil physical, chemical and biochemical properties. However, the mechanisms of termite mound turnover and the effects of such turnover on temporal and spatial patterns of nutrients in surrounding soils have rarely been studied. Here, we investigated the relationship between the stabilization and redistribution of soil nutrient properties and the chronological development of termite mounds (primary, secondary-occupied and abandoned mounds). The study was conducted to assess the concentrations of nutrients and water content in the biogenic structures produced by two fungus-growing termites (*M. annandalei* and *O. yunnanensis*) in different mound stages. Samples were collected along a transect at regular intervals proportional to the size of the biogenic structure. We found the concentration of NO_3^- in the following sequence: active mound, abandoned mound, and the surrounding soils. The concentrations of organic carbon (C_{org}) and total N in the active mounds were significantly lower than those in the surrounding topsoils, although a weak difference was observed with subsoils. The concentrations of C_{org} and total N in the abandoned mounds were significantly higher than those in active mounds, whereas no difference was observed with surrounding soils. The mean concentrations of nutrients were significantly different between fresh parts (sampling locations 1–3) and old parts (locations 4–6) of the secondary-occupied mounds. The nutrients in the fresh parts of the mound approached the values in the primary mound; whereas the nutrients in the old part of the mound approached the values in the abandoned mound. Our results indicate that nutrient concentrations change through the chronological development of termite mounds, which are hot spots of nutrients that subsequently affect ecosystem processes at specific spatial and temporal scales through the stabilization and redistribution of nutrients.

1. Introduction

Termites (Isoptera) are often considered major ecosystem engineers that alter soil properties through the selection, translocation and ingestion of organic and inorganic materials (Holt and Lepage, 2000; Sarcinelli et al., 2009; Levick et al., 2010). Termite mounds built by fungus-growing termites, which have a dominant role in tropical ecosystems, are numerous in the tropical forests of southeast China, particularly in areas converted to rubber plantations. Although many studies demonstrate that termites enrich the inside of their mounds with carbon, nitrogen, clay and exchangeable cations compared with the adjacent soils unmodified by termites (Mujinya et al., 2010; Seymour et al., 2014), the mechanisms of termite mound turnover and the effects of mound turnover on temporal and spatial patterns of nutrients in surrounding soils have rarely been studied for this region.

The effect of termites on the physical properties of soils, particularly the effects of fungus-growing species, is primarily related to burrowing and mounding activities through which the soil chemical and biochemical characteristics are also modified compared with original soils (Darlington et al., 1997; Mahaney et al., 1999; Mills et al., 2009). Fungus-growing termites build mounds constructed of a mixture of soil particles and saliva and also provide a protective enclosure around

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https://doi.org/10.1016/j.soilbio.2017.11.018

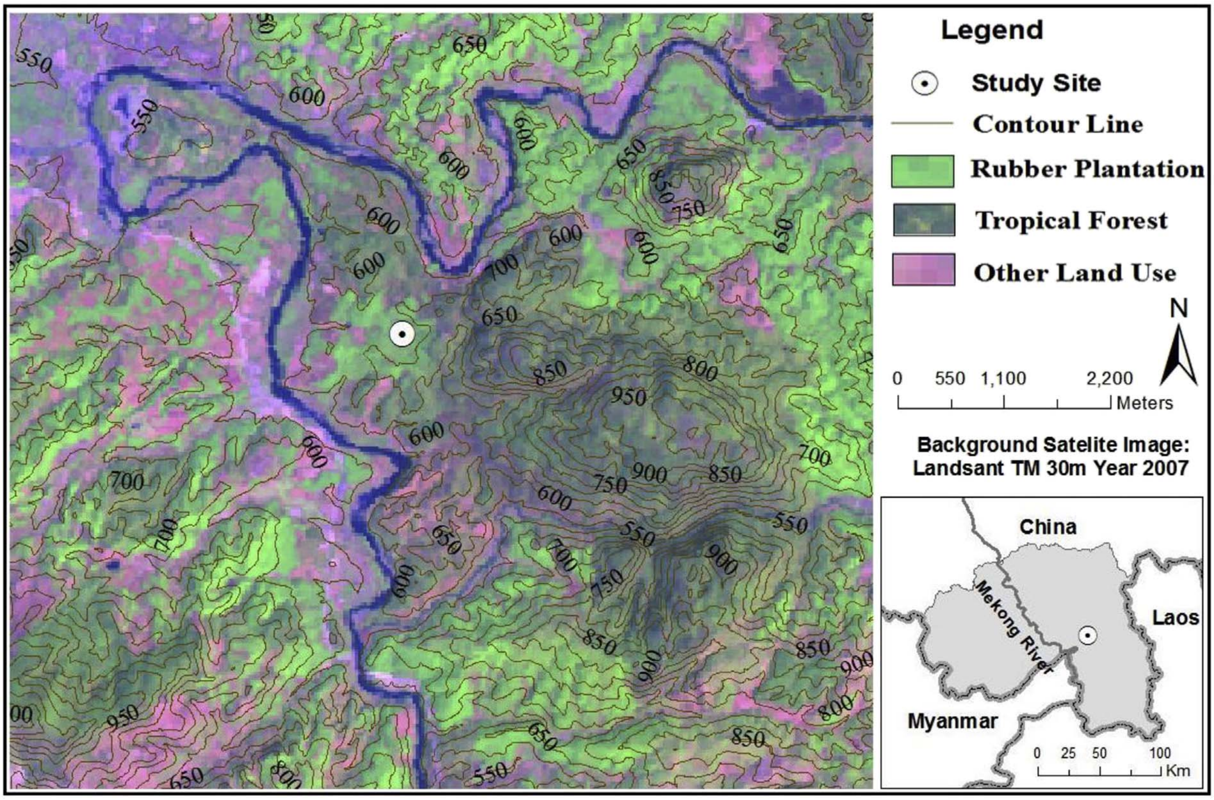

Fig. 1. Map showing location of the study site (21°55′39″N, 101°15′55″E) in Yunnan Province, southwest China.

potential food items within which feeding and foraging take place (Bagine, 1984). In general, termite mounds have increased levels of nutrients, including enrichment of N-based compounds (Seymour et al., 2014), phosphorus (Rückamp et al., 2010), and micronutrients (Seymour et al., 2014). In the mound structures of fungus-growing termite species, the quantity and quality of soil organic matter (SOM) are variable depending on the initial soil properties and on the specific species.

However, because of the low stability of termite mounds and the high rainfall in the study area, these types of biogenic structure (BS) are quickly recycled in soils. A decrease in nutrient contents may occur in the older or dead mounds due to weathering and organic matter decomposition or the activities of termites (Cammeraat et al., 2002; Roose-Amsaleg et al., 2005). For example, Congdon et al. (1993) and Schwiede et al. (2005) reported leaching of nitrates and ammonium from termite mounds into the surrounding soil. Moreover, recolonization of dead mounds is very common in populations of *Macrotermes*. Previous studies demonstrate that other colonies may occupy a mound after the death of the original builders, and these recolonizers may be the same or a different species or both (Pomeroy, 1976). Rückamp et al. (2009) found higher nitrate and ammonium concentrations and lower carbon fluxes in soil water leaching from older mounds inhabited by secondary termites than in water from mounds inhabited by primary termites or reference soils.

Because the activities of termites and environmental weathering both play important roles in nutrient cycling processes in tropical soils, which are notoriously nutrient deficient environments (Cerdà and Jurgensen, 2011; Menichetti et al., 2014), some researchers are attempting to understand the mechanisms and the evolution of termite mounds according to changes in nutrient distributions. For example, Menichetti et al. (2014) compared the concentrations of nutrients and biochemical activity of colonized and abandoned mounds of the litter-feeding termite (*Macrotermes* spp.) located in the Borana District, Ethiopia. They found that the abandoned termite mounds had higher microbial biomass and activity and displayed a redistribution of nutrients compared with the adjacent soils, whereas weakly significant differences were detected compared with mounds colonized by termites. Additionally, Rückamp et al. (2012) examined the chemical soil properties in and below mounds that were built by the primary termite (*Cornitermes silvestrii*) and the secondary colonizer (*Nasutitermes kemneri*) in tropical savannahs. The highest enrichment of chemical soil properties compared with the control soil was found in the mature or primary inhabited mounds, whereas the enrichment declined in older mounds but remained higher than that in the control soils.

Nevertheless, we are unaware of any studies that have been conducted on comparisons or relationships among the different stages or ages of mounds (i.e., the primary, secondary and abandoned mounds) in tropical soils, which are notoriously nutrient deficient environments (Garba et al., 2011; Miyagawa et al., 2011). Moreover, in the assessment of nutrient distributions, the structure of the mound as a whole is normally considered (Verchot et al., 2003; Holec et al., 2006), and few researchers have investigated different parts of the structure, particularly those of the secondary mounds with drastically varied physical and chemical properties in different parts of the structure. These omissions prevent obtaining a better understanding of the magnitude and persistence of termite-induced effects on soil primary properties and functions and in general, on the evolution of termite mounds. Thus, the goals of our study were to (1) assess the spatial and temporal variability of organic matter and nutrient contents of the biogenic structures produced by termites; (2) understand the effect of two dominant termite species feeding guilds on soil genesis in the Xishuangbanna area; and (3) evaluate the contents of organic matter and nutrients as indicators of chronological development in different states or ages of mounds, i.e., the primary, the secondary-occupied and the abandoned mounds.

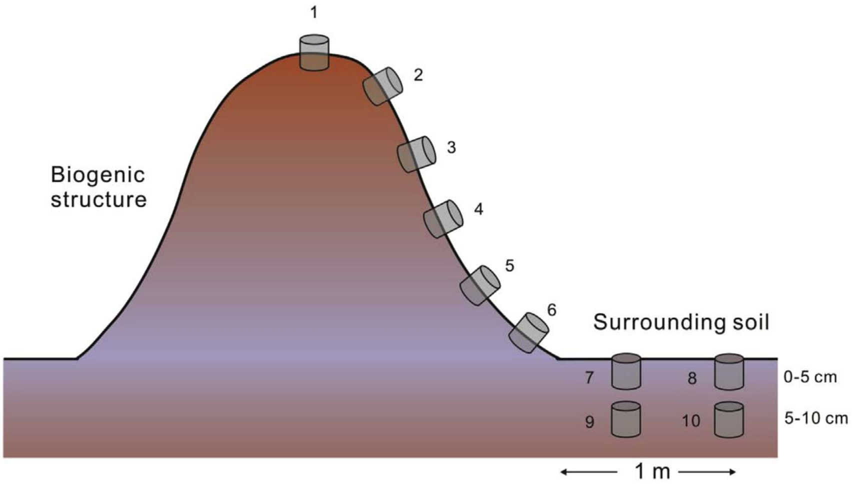

Fig. 2. Schematic diagram illustrating the sampling methodology to collect samples from the biogenic structures. Sample locations 1–6 were evenly located throughout the biogenic structure, and the distance between locations 6 and 7 was equal to the distance between 5 and 6. Sample location 9 was taken 1 m from the biogenic structures in 0–5 cm depth, while sample locations 8 and 10 were taken in 5–10 cm depth.

2. Materials and methods

2.1. Study site

The experiment was conducted in an artificial rubber plantation catchment (ARPC) in the Xishuangbanna Tropical Botanical Garden (XTBG; 21°55′39″ N, 101°15′55″ E) of Yunnan Province in southwestern China, which is approximately 800 km from the Bay of Bengal to the southwest (Fig. 1). The region has a strongly seasonal climate with two distinct seasons alternating during the year, namely, the rainy season (from May to October) and the dry season (from November to April). The tropical southern monsoon from the Indian Ocean dominates the climate during the rainy season, whereas the southern edges of the subtropical jet streams prevail and deliver the dry and cold air during the dry season. The mean annual rainfall ranges from 1500 to 1800 mm of which 80–90% of the total rainfall is concentrated in the rainy season. The annual average temperature in this region varies from 24 to 29 °C. The mean monthly relative humidity is always high, between 75% and 80%.

Soils under the rubber tree monoculture are approximately 2 m deep and well drained with a clay loam texture (42% coarse sand, 34% silt and 24% clay). The dominant soil type in the landscape is classified as laterites (Oxisols) developed from arenaceous shale sediments with approximately 2 m of depth. The parent material at a depth of 2 m consists of a 30–40 cm thick layer of gravel deposited by a distributary of the Mekong River. The rubber monoculture (clone PB86) plantations were arranged into double rows that were planted at a density of 2 × 4.5 m and separated by 16-m-wide gaps between rows. A commercial fertilizer containing N, P and K was point-applied in March and August at a dose of approximately 0.1 kg N per tree hole per year in each study site (Li et al., 2012).

2.2. Sample collection

The study was conducted during April 2016 at the end of the dry season. The entire study area had a rich diversity of epigeic mounds constructed by various termite species at a density of 102 ha^{-1}. We collected samples from two types of abundant and conspicuous surface mounds produced by *Macrotermes annandalei* and *Odontotermes yunnanensis* (Isoptera, Macrotermitinae); both species play dominant roles in the study area. To identify the different states of mounds, i.e., the primary, the secondary-occupied and the abandoned mounds, we distinguished between mounds in the building and weathering phases based on the presence or absence of both worker and soldier castes of the termites and also on the degree of weathering. Mounds containing both castes of termite represented the building phase, whereas the absence of termites and the presence of exposed runways or an eroded, collapsed structure represented the weathering phase. We examined three mounds that looked fresh and were primarily inhabited by *M. annandalei* and three mounds which were primarily inhabited by *O. yunnanensis*. We also examined four abandoned mounds (the termite species of the abandoned mounds were not identified because of the long time periods with the termites absent), which were weathered and overgrown by mosses and other plant species. In addition, we examined secondary-occupied mounds that were reconstructed from abandoned mounds and partly inhabited by the same or different termite species as secondary inquilines, i.e., three secondary-occupied mounds inhabited by *M. annandalei* and three secondary-occupied mounds inhabited by *O. yunnanensis*. The mounds were randomly arranged in the study area and each mound was separated by a minimum of 40 m. The mounds constructed by these two species of termites were almost identical with orange or yellowish domes. The mounds were constructed of small soil particles and were approximately 2.5 m wide and 1.5 m high for primary and secondary-occupied mounds and approximately 2.6 m wide and 0.8 m high for abandoned mounds.

The sample procedure of the biogenic structure corresponded to the method used by Jiménez et al. (2006, 2008), which is indicated in Fig. 2. Soils for chemical analysis were collected along an imaginary transect line throughout the biogenic structure from the top centre to the bottom edge of the biogenic structure at the soil surface. Specifically, for each termite mound, three subsamples of 0.5 kg each were taken using a metal cylinder (10 × 5 cm) from three points following a circle around the mound and were evenly separated with seven locations along the transect, i.e., samples 1–6 corresponded to the biogenic structure and 7 to the surrounding soil. Then the three subsamples around the same circle were mixed into a 1.5 kg sample. An additional sample 8 was taken at 1 m distance from the termite mound. Samples 7, 8 and 9, 10, which were taken to 10 cm and subdivided into 0–5 and 5–10 cm, represented the control soils (surrounding soils). Remarkably, the secondary-occupied termite mounds had a special structure, because the fresh part was significantly different from the old part; therefore, we collected samples 1–3 evenly located in the fresh part and samples 4–6 evenly located in the old part. All field replicate samples were placed separately in identifiable plastic bags, stored in an ice chest to halt possible further mineralization processes, and shipped to the analytical laboratory immediately after sampling.

Inorganic N concentrations, NH_4^+ and NO_3^-, were determined by colorimetric methods (Baker et al., 1975). The elemental C and N concentrations were measured by dry combustion with a Vario MAX CN elemental analyser (Langenselbold, Germany). The soil in this area was free of inorganic C because no reaction occurred when HCl was added to the soil; therefore, all C measured was considered equivalent to the

SOC (Chivenge et al., 2011). The soil moisture content (%) was measured by weighing each soil sample before and after oven drying at 105 °C for 24 h.

2.3. Soil analyses

The soil physical-chemical properties, NH_4^+, NO_3^-, C_{org}, and total N, were analysed on aggregated samples. The data of primary mounds and secondary mounds built by each termite species are the mean of three independent replicates, and the data of abandoned mounds are the mean of four independent replicates. The *F*-test was used for comparisons among sampling locations when significant differences were found. Because the termite species of abandoned mounds were not identified, we conducted two separate MANOVAs. A three-way MANOVA (general linear model) was performed with species, mound type (except for abandoned termite mounds) and sample location as main fixed factors for the termite mounds constructed by *M. annandalei* and *O. yunnanensis*, and a two-way MANOVA was performed with mound type (including abandoned termite mounds) and sample location. Because no significant differences were found for the physical-chemical properties (except for NH_4^+) between the two termite species mounds in the same mound stage, we considered the structures as a whole to compare the differences in the physical-chemical properties among the primary mounds, secondary mounds, abandoned mounds and the surrounding soils. Then, the *F*-test was used for comparisons among mound stages when significant differences were found. A correlation analysis was conducted to determine the relationships among each variable of physical-chemical properties in the termite mounds built by the two termites species.

3. Results

3.1. NO_3^- and NH_4^+ concentrations in biogenic structures

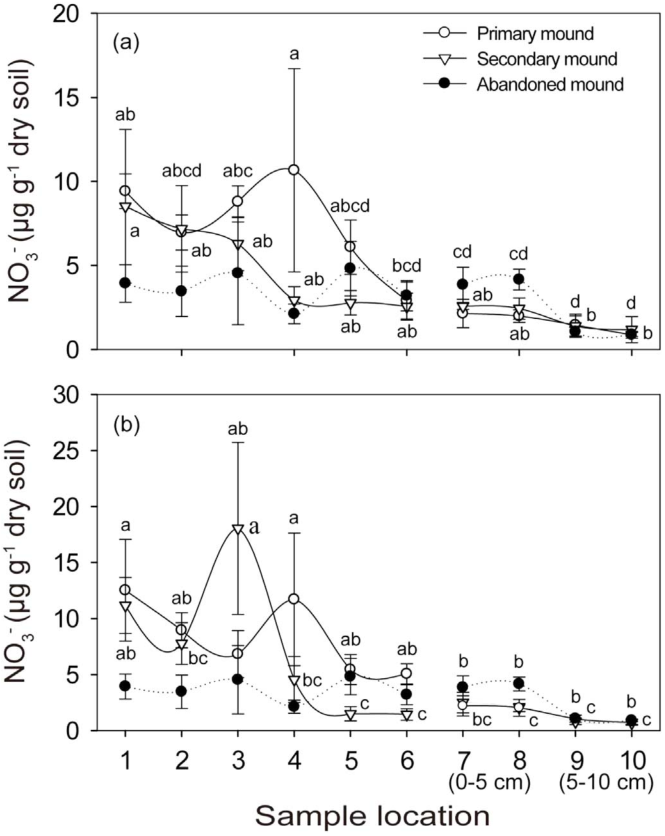

Fig. 3. NO_3^- concentrations in the biogenic structures of *M. annandalei* (a) and *O. yunnanensis* (b) with three types of mound (including primary mound and secondary occupied mound, and abandoned mound). Error bars are SE. Means with different lowercase/capital letters between sampling locations are significantly different ($P < 0.05$). The lowercases above the curve correspond to the primary mound, the capital letters above the curve correspond to the abandoned mound, while the lowercases under the curve correspond to the secondary occupied mound. See Fig. 1 for details of sampling scheme.

For the biogenic structures (BSs) built by *M. annandalei*, mound type and sample location had significant effects on the NO_3^- concentrations ($F_{\text{mound type}} = 3.31, P = 0.042$; $F_{\text{sample location}} = 4.75, P < 0.001$), and a significant independent and interactive effect of mound type and sample location on NO_3^- concentrations was detected for the BSs built by *O. yunnanensis* (Table 1). NO_3^- concentrations in both primary and secondary mounds built by the two termite species showed significant differences among sample locations. However, the change in NO_3^- concentrations in abandoned mounds was slight, with the differences among sample locations inconspicuous (Fig. 3a and b). The highest NO_3^- concentration in the primary mounds built by *M. annandalei* was in location 4 with 10.66 µg g dry soil^{-1}, and the lowest concentration was in location 6 with 2.92 µg g dry soil^{-1} (Fig. 3a). The NO_3^- concentration in the secondary-occupied mound built by *M. annandalei* decreased along the BS and ranged from 8.522 to 2.57 µg g dry soil^{-1} for the 1 to 6 distance (Fig. 3a). Consistent with the mounds built by *M.*

Table 1
Result of a general linear model testing the effects of termite species, mound types and sample locations on NO_3^-, NH_4^+, C_{org}, and total N, as well as moisture content (by a MANOVA).

Tested effects	d.f.	NO_3^-		NH_4^+		C_{org}		Total N		Soil moisture	
		F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
(A) Species	1	1.45	0.232	169.57	< 0.001 ***	0.4	0.526	0.001	0.972	0.35	0.557
Mound types	1	1.73	0.192	0.16	0.693	16.56	< 0.001 ***	8.69	0.004 **	15.7	< 0.001 ***
Locations	9	9.7	< 0.001 ***	0.96	0.478	42.29	< 0.001 ***	24.07	< 0.001 ***	170.58	< 0.001 ***
Species × Mound types	1	0.25	0.619	4.56	0.036 *	16.09	< 0.001 ***	22.148	< 0.001 ***	31.88	< 0.001 ***
Species × Locations	9	0.59	0.798	0.33	0.963	0.31	0.97	0.289	0.976	0.92	0.511
Mound types × Locations	9	1.7	0.103	0.29	0.975	2.06	0.043 *	1.366	0.217	7.68	< 0.001 ***
Species × Mound types × Locations	9	1.00	0.449	0.58	0.808	1.86	0.071	1.367	0.217	3.00	0.004 **
(B) Mound types	2	3.31	0.042 *	12.95	< 0.001 ***	38.63	< 0.001 ***	32.867	< 0.001 ***	211.4	< 0.001 ***
Locations	9	4.75	< 0.001 ***	0.41	0.925	20.94	< 0.001 ***	14.14	< 0.001 ***	80.69	< 0.001 ***
Mound types × Locations	18	1.33	0.198	0.57	0.911	1.88	0.032 *	1.313	0.207	13.49	< 0.001 ***
(C) Mound types	2	3.82	0.027 *	30.2	< 0.001 ***	26.54	< 0.001 ***	17.124	< 0.001 ***	170.22	< 0.001 ***
Location	9	6.59	< 0.001 ***	0.72	0.692	16.87	< 0.001 ***	10.01	< 0.001 ***	89.57	< 0.001 ***
Mound types × Locations	18	2.42	0.004 **	0.33	0.995	1.2	0.286	0.548	0.923	11.5	< 0.001 ***

F-values and significance are reported. The results including the following: (A) The effects on two species and two mound types (including primary mound and occupied mound); (B) The effects on the mound of *M. annandalei* and three mound types (including primary mound and occupied mound, and abandoned mound); (C) The effects on the mound of *O. yunnanensis* and three mound types (including primary mound and occupied mound, and abandoned mound).

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

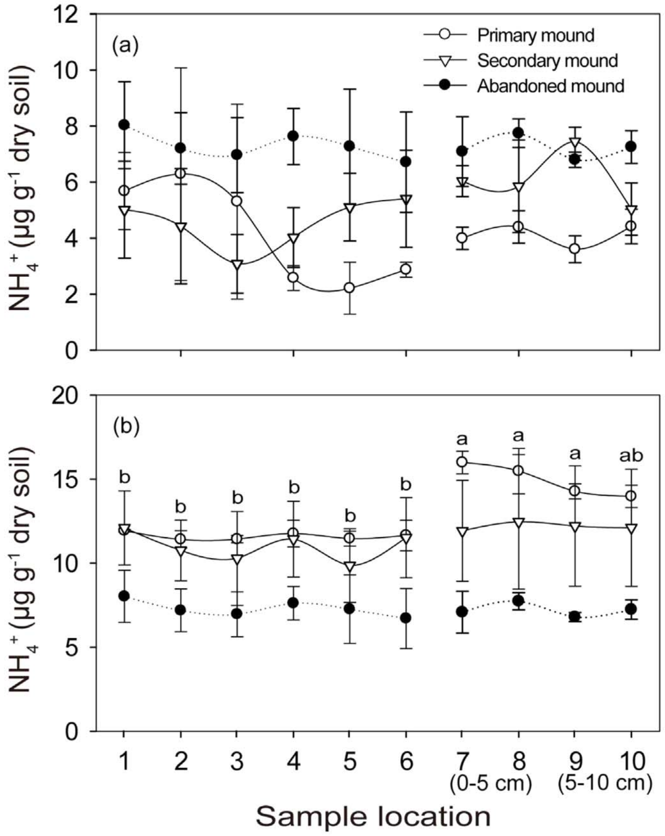

Fig. 4. NH_4^+ concentrations in the biogenic structures of *M. annandalei* (a) and *O. yunnanensis* (b) with three types of mound (including primary mound and secondary occupied mound, and abandoned mound). See Fig. 2 for abbreviation.

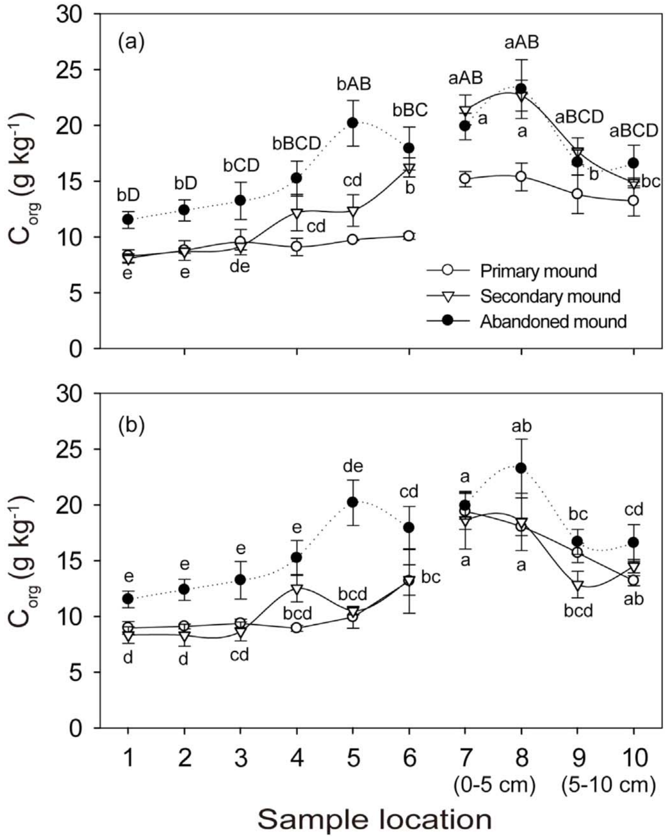

Fig. 5. Concentrations of C_{org} in the biogenic structures of *M. annandalei* (a) and *O. yunnanensis* (b) with three types of mound (including primary mound and secondary occupied mound, and abandoned mound). See Fig. 2 for abbreviation.

annandalei, NO_3^- concentrations in both the primary and secondary-occupied mounds built by *O. yunnanensis* exhibited significant locational variation and ranged from 12.53 to 5.06 μg g dry soil^{-1} and 18.04 to 1.44 μg g dry soil^{-1}, respectively (Fig. 3b).

NH_4^+ concentrations were significantly different between termite species ($F = 169.57$, $P < 0.001$). The differences in NH_4^+ concentrations between BSs built by *M. annandalei* and the surrounding soils were inconspicuous and ranged from 6.29 to 2.21 μg g dry soil^{-1} and from 7.45 to 3.09 μg g dry soil^{-1} in the primary and secondary mounds, respectively (Fig. 4a). In the BSs produced by *O. yunnanensis*, the NH_4^+ concentrations were statistically lower in the primary mound than in surrounding soils, but no differences occurred in the secondary mound, with NH_4^+ concentrations that ranged from 16.00 to 11.41 μg g dry soil^{-1} in the primary mound and from 12.46 to 9.86 μg g dry soil^{-1} in the secondary mound (Fig. 4b).

3.2. C_{org} and total N concentrations in biogenic structures

Mound type and sample location had significant effects on C_{org} concentrations ($F_{\text{mound type}} = 16.56$, $P < 0.001$; $F_{\text{sample location}} = 42.29$, $P < 0.001$), but C_{org} was not affected by termite species (Table 1). In the BSs produced by *M. annandalei*, a significant independent and interactive effect of mound type and sample location was detected on C_{org} concentrations ($F_{\text{mound type}} = 38.63$, $P < 0.001$; $F_{\text{sample location}} = 20.94$, $P < 0.001$; $F_{\text{mound type*sample location}} = 1.88$, $P = 0.032$). Mound type and sample location had significant effects on the C_{org} concentrations in the BSs built by *O. yunnanensis* ($F_{\text{mound type}} = 26.54$, $P < 0.001$; $F_{\text{sample location}} = 16.87$, $P < 0.001$). The differences in C_{org} concentrations in the primary mound produced by *M. annandalei* were inconspicuous, whereas in the secondary-occupied mound, sample locations 1 to 3 were significantly lower in concentration than that at sample locations 4 to 6. C_{org} concentrations in the abandoned mound, with values that ranged from 20.19 to 11.53 g kg dry soil^{-1}, were much higher than those in the primary mound (10.07–8.31 g kg dry soil^{-1}) and in secondary-occupied mound (16.24–8.07 g kg dry soil^{-1}) produced by *M. annandalei* (Fig. 5a). C_{org} concentrations in both primary and secondary-occupied mounds built by *O. yunnanensis* increased with sampling distance and ranged from 13.18 to 8.93 g kg dry soil^{-1} and 13.28 to 8.31 g kg dry soil^{-1}, respectively (Fig. 5b).

Similar to the trend with C_{org} concentrations, mound type and sample location had significant independent effects on total N concentrations ($F_{\text{mound type}} = 8.69$, $P < 0.01$; $F_{\text{sample location}} = 24.07$, $P < 0.001$), but no effect of termite species on N concentrations was detected (Table 1, Fig. 6a and b). Mound type and sample location had significant independent effects on N concentrations in the BSs built by those two termite species.

3.3. Soil moisture

For water content, a significant independent and interactive effect was detected for mound type and sample location, but the effect of termite species was not significant ($F_{\text{mound type}} = 15.7$, $P < 0.001$; $F_{\text{sample location}} = 170.58$, $P < 0.001$; $F_{\text{mound type*sample location}} = 31.88$, $P < 0.001$). The water content in abandoned mounds was not significantly different among the sampling locations, with values that ranged from 24.40% to 20.45%. However, the water content in abandoned mounds was significantly higher than that in the primary and secondary-occupied mounds produced by *M. annandalei*, which ranged from 17.11% to 5.79% and from 22.15% to 4.42%, respectively (Fig. 7a). The water content at sampling locations 1 to 3 in secondary-occupied mounds was significantly higher than that at sampling

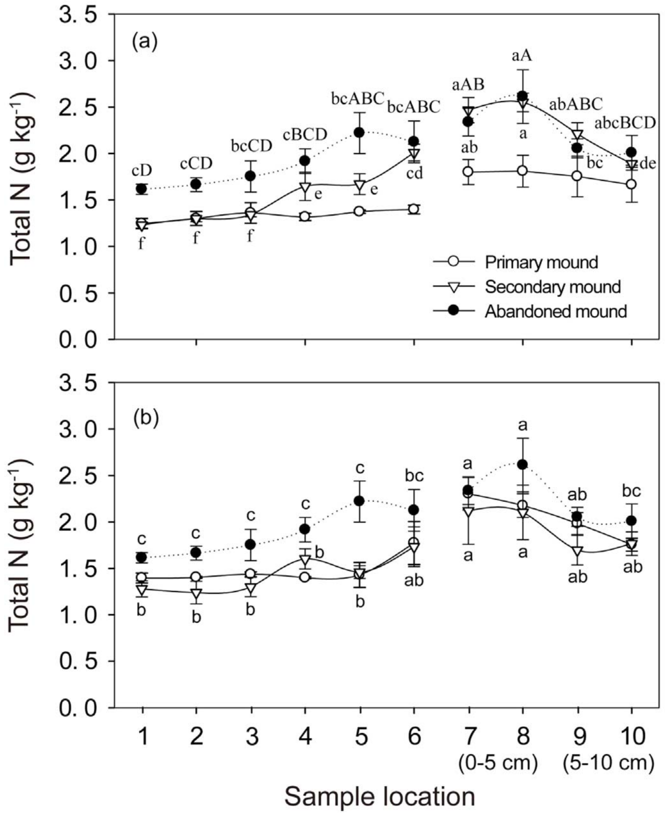

Fig. 6. Concentrations of N in the biogenic structures of *M. annandalei* (a) and *O. yunnanensis* (b) with three types of mound (including primary mound and secondary occupied mound, and abandoned mound). See Fig. 2 for abbreviation.

locations 4 to 6. The water content increased with sampling distance, and values at the location of sample 6 approached those obtained in the surrounding soil.

3.4. Biogenic structures and control soils

To compare the nutrient distributions of different stages of the mounds and the control soils, the nutrient concentrations in the biogenic structures as a whole were assessed in different stages. For *M. annandalei*, the NH_4^+ concentrations in the adjacent soils were significantly higher than those in both primary and secondary mounds, whereas no significant differences were observed between soil and the abandoned termite mound (Fig. 8c). However, for *O. yunnanensis*, the NH_4^+ concentrations in the abandoned mound were significantly lower than those in the two other mound stages and in the control soils (Fig. 8d). The NO_3^- concentrations in the primary mounds by both *M. annandalei* and *O. yunnanensis* were significantly higher than those in the secondary mounds and were higher than those in the abandoned mounds (Fig. 8a and b). The trends for C_{org} and total N concentrations and C:N values were similar in different mound stages and in control soils. Specifically, those factors in primary mounds built by the two termites species were not different from those in secondary termite mounds; however, these factors were significantly lower in both the abandoned mounds and control topsoils (Fig. 8a–j). In addition, all the physical and chemical properties (i.e., NO_3^-, NH_4^+, C_{org}, total N and soil moisture) of each termite mound built by the two termites species were correlated at different levels (Fig. S1).

4. Discussion

The comparison of our results with those reported in other studies was limited, because only Jiménez et al. used the same sampling methodology as in this study (Jiménez et al., 2006, 2008), and no other studies in the literature were available. However, some general considerations about the spatial and temporal variability of nutrient contents can be discussed. In our study, no differences were detected for C_{org} or total N concentrations between *M. annandalei* and *O. yunnanensis* mounds. Jouquet et al. (2005) found a similar result, and the C_{org} and total N contents in the mounds built by *Ancistrotermes* did not differ from those in the mounds built by *Odontotermes* in a West African savannah. Although Mora et al. (2003) did not find any differences in the C_{org} concentrations in the mounds built by termites within the same genus (*Odontotermes*) that were sampled in the Thiès region, differences were found in a mongo orchard soil with higher organic matter content. This contrast could indicate that the degree of C_{org} concentration in a termite mound depends more upon the richness of the organic matter in the adjacent soil than on the species of termite. However, termite species had a conspicuous effect on NH_4^+ concentrations, because significantly higher NH_4^+ concentrations were found in the mound structures inhabited by *O. yunnanensis* than in the mounds built by *M. annandalei*, although a similar difference was not observed for NO_3^- concentrations. This difference between species indicated that the ammonification rate was higher in *O. yunnanensis* mounds than that in *M. annandalei* mounds.

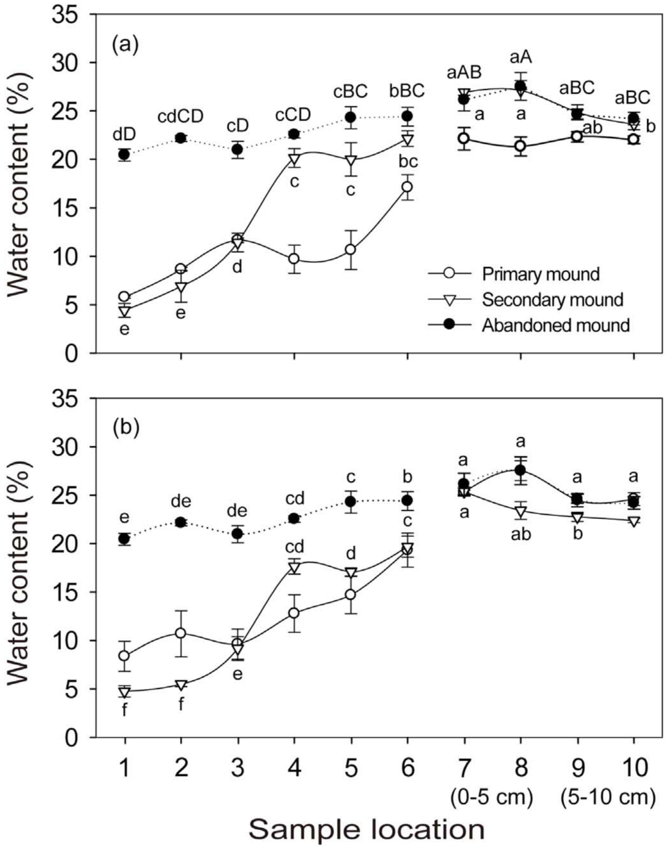

Fig. 7. Moisture contents in the biogenic structures of *M. annandalei* (a) and *O. yunnanensis* (b) with three types of mound (including primary mound and secondary occupied mound, and abandoned mound). See Fig. 2 for abbreviation.

Although enriched in nitrate, with NO_3^- nearly 3-fold more concentrated than that in the surrounding soils, mounds were not similarly enriched in ammonia (Figs. 3 and 8). Seymour et al. (2014) found similar results in which termitaria of *Diospyros mespiliformis* were enriched in nitrates but not ammonium. However, the results of López-Hernández (2001) are in contrast, with the mounds of *Nasutitermes ephratae* enriched in ammonia but not nitrates. The differences are most likely due to mound pH, because the mounds sampled by López-Hernández (2001) were acidic (mean pH = 5.6), whereas the mounds

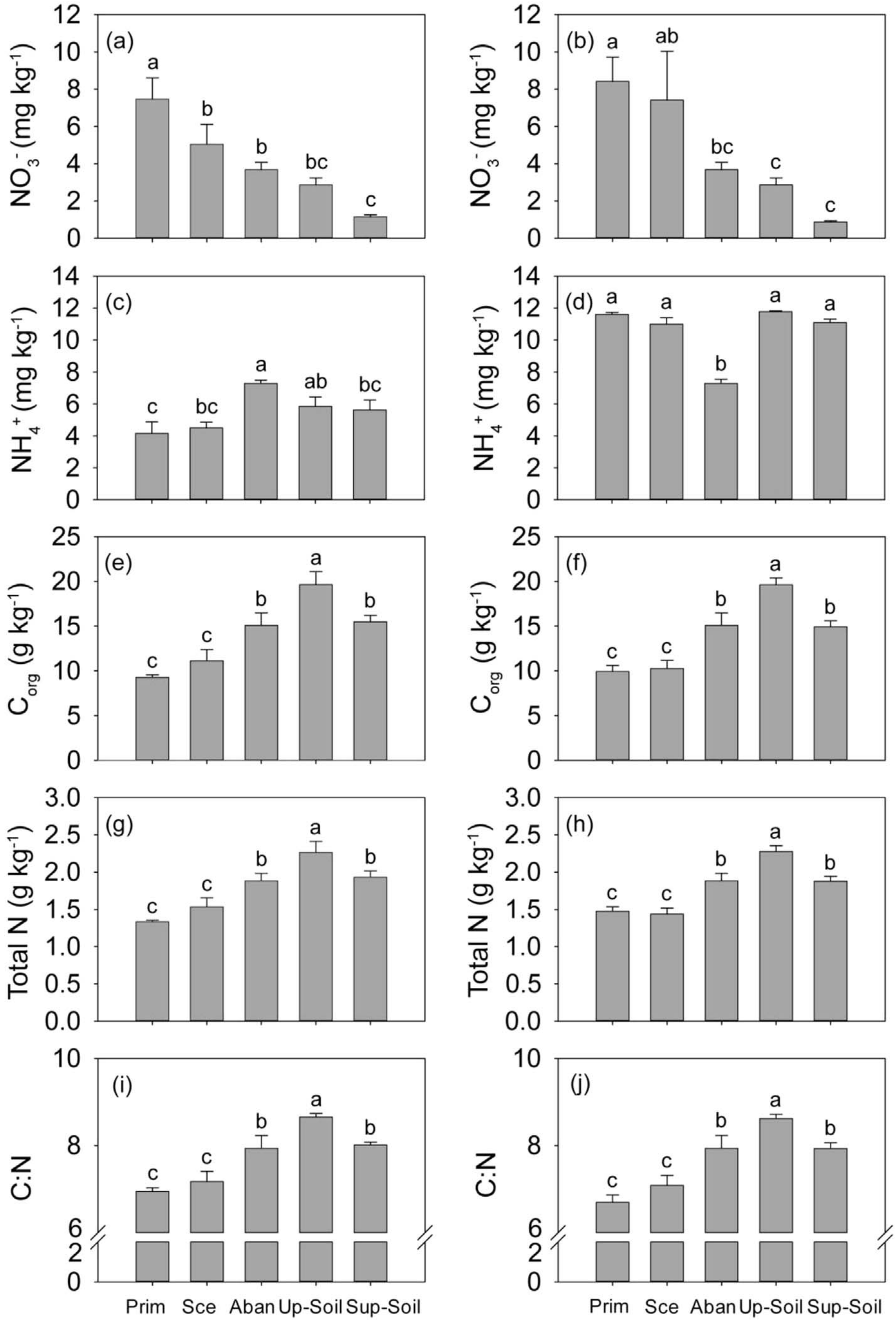

Fig. 8. Nutrient values in mound structures (primary, abandoned and secondary-occupied mounds) and adjacent soils. NH_4^+ and NO_3^- concentrations, as well as concentrations of C_{org} and N in the mounds of *M. annandalei* and the adjacent soils (a, c, e, g), and in the mounds of *O. yunnanensis* and the adjacent soils (b, d, f, h). Error bars are SE. Means with different lowercase letters are significantly different ($P < 0.05$). Prim, primary mounds; Sec, secondary-occupied mounds; Aban, abandoned mounds; Up-Soil, 0–5 cm depth of surrounding soils; Sup-Soil, 5–10 cm depth of surrounding soils.

were alkaline in studies of Seymour et al. (2014) and in our study, with mean pH = 7.6 and 7.1, respectively. Within acidic environments, the absence or lack of the bacteria responsible for the reaction results in nitrification proceeding slowly. As a consequence, in acidic soils, ammonium is usually dominant, whereas in neutral or alkaline soils, nitrate is dominant (Seymour et al., 2014).

Lower amounts of organic matter and total N were found in the primary or young termite mounds than in the surrounding topsoils. Previous comparisons of the concentrations of organic matter between young mounds and the soil matrix have variously found no effect (Eschenbrenner, 1986), enrichment in mounds (Jouquet et al., 2003) or a decrease in organic matter in mounds relative to the surrounding topsoil (Brauman et al., 2000; Fall et al., 2001; Jouquet et al., 2005). This variability of the response of soil properties to termites is likely a function of termite foraging habits and mound building (López-Hernández et al., 2006; Chapuis-Lardy et al., 2011). Therefore, the specific species of termites likely influenced the relative deterioration in this study area. For example, termites are well documented to use different strategies depending on whether the structure to be built is permanent (a nest) or temporary (crop sheeting and crop galleries) (Jouquet et al., 2002). In our study, species of these genera select finer particles from subsoil (exceeding 60 cm in depth) to build their mounds, and therefore the mounds of these termites contain little organic matter compared with the adjacent soils, because the concentration of organic material decomposed from plant root and leaf residue is much lower in deep soil. Similar research was conducted by Jiménez et al. (2008), and they found that organic matter concentrations were significantly lower in the biogenic structures of *A. laevigata* than in surrounding soils in Colombian savannahs and pastures, which indicated that the conspicuous structures of termites were transported from deep horizons and did not undergo significant modification. Alternatively, the trophic and mound construction behaviours of termites can also contribute to the low carbon contents (Makonde et al., 2015). Indeed, a previous study indicates that the fungus-growing termites build their mounds using soil and clay cemented by salivary secretions, which results in mounds enriched with clay particles but impoverished in carbon and nitrogen (Harry et al., 2001). As another hypothesis, the decrease of organic matter and poor soil nutrient content could have originated from the limitation of plant biomass accumulation on the termite mounds. Ackerman et al. (2007) hypothesized that poor conditions in termite mounds, such as stronger mechanical resistance to root penetration, low soil water capacity, elevated clay content and poor soil nutrient content, resulted in low root biomass, poor seed germination and slow seedling growth, compared with adjacent soils. We found similar conditions in the active termite mounds, with high mechanical resistance to penetration, low water content (average 10%), and acidity (7.1), which are severely limiting to the growth of mosses or plants.

The concentrations of NO_3^- declined significantly following the transformation from primary mound to abandoned mound. However, the concentrations of NO_3^- were higher in the soil surrounding the abandoned mound than in the soils surrounding the primary mound, which could be attributed to either termite activities or a depletion of the nitrate in the aboveground part of the mound (Figs. 3 and 8). Nutrient enrichment of mounds largely depends on preferential selection and transportation of clay, calcite, ground water and organic matter by termites during mound construction (Petts, 2009). Nutrients temporarily withheld in termite mounds are eventually returned to the soil surface by erosion (Sileshi and Arshad, 2012). Schwiede et al. (2005) proposed that nutrient redistribution after mound abandonment was primarily due to the leaching and erosion that continued until the complete disappearance of the chemical gradient created during the mound life cycle, resulting in a decrease in nutrients in abandoned mounds and an increase in the surrounding soils. Our results are also supported by the study of Rückamp et al. (2009) who reported larger differences in dissolved organic carbon (DOC) and NO_3^--N in older mounds re-colonized by secondary termites than in younger mounds inhabited by primary populations. Thus, the hypothesis is supported that the aging of the mounds is a primary factor influencing nutrient distribution in the soils colonized by termites. In contrast to NO_3^-, the concentrations of C_{org} and N in the abandoned mounds were significantly higher than those in the primary mounds, whereas the concentrations were weakly significantly lower than those in the surrounding soils (Figs. 5, 6 and 8). The differences in C_{org} and N contents occurred among different mounds could be attributed to invasion and enrichment of mosses and other plant species or to changes in fungal communities. For plant cover, Smith and Yeaton (1998) found that the cover and density of grass (the species with low productivity and low nutrition requirements) on abandoned or eroded mounds of *Trinervitermes trinervoides* declined. However, grass (the species with high nutrition requirements) and shrubs increased compared with active mounds, resulting in increased concentrations of Mg, Ca, N, P, and total exchangeable cations (T.E.C.), in addition to increases in protein and nitrogen contents in the plants grown on soils from the abandoned mounds. For changes in the fungal community, Diouf et al. (2006) found that the characteristics of fungal communities of the fresh sheetings of termites were remarkably different from those in the old ones, which were similar to those observed in the surrounding soil.

Notably, although the mean concentrations of NH_4^+ did not differ between young parts (locations 1–3) and old parts (locations 4–6) of the secondary-occupied mounds, the mean concentrations of NO_3^-, C_{org}, and N and water content were significantly different between the two parts of the mound, because these factors changed drastically from location 3 to 4 (Figs. 3, 5 and 6). These results indicated that the old parts of the mound were not used by secondary termite colonies and that the galleries were rebuilt. Additionally, soil material of the young parts was derived from soil at great depths or presumably over great distances. Likewise, the lowest contents of C_{org} and soil water were recorded in the young parts of secondary-occupied termite mounds in which the values were similar to those of the primary mounds; whereas the highest contents of the same factors were found in the old parts, which approached the values of the abandoned mounds. The implications of these results are that the young parts of mounds inhabited by the secondary termites were rebuilt in the centre of the abandoned termite mounds, and that the original mounds erected by the primary mound builders were not used directly.

The water content in the colonized mounds was significantly lower than that in abandoned mounds, with the water content of the abandoned mounds different from that of the surrounding soils (Figs. 7 and 8). The differences in water content occurred among different mounds mostly because we collected the samples in the dry season, and the surrounding soils in this region contained high water content due to the supply of fog. A previous study showed that the absolute average amount of annual fog water dripping was 89.4 mm, with 86% occurring in the dry season in which the fog water could maintain high moisture in the environment (Liu et al., 2004). However, the water content in the colonized mounds was low because the termites must keep the mounds dry and tough to prevent the roots of surrounding plants invading into the mounds and then taking up the concentrated stores of nutrients that are often held in the bases of termite mounds. By contrast, many observations demonstrate that the harvesting activity of termites creates a dense network of underground galleries that are connected to the soil surface by foraging holes through which water can penetrate and that constitute preferential flow paths (Léonard and Rajot, 2001; Turner et al., 2006).

5. Conclusions

The effects of termite activities and environmental factors on the chronological development of termite mounds can be stated as three hypotheses (Fig. 9). First, termites build their mounds using deep soils, causing lower contents of organic carbon and nitrogen in the primary mounds than in adjacent soils. However, more N-compounds were observed in the primary mounds because of the active microbial communities, which could be selectively activated due to the high protein content of termite saliva. Second, the mounds may be abandoned for a variety reasons, causing the invasion and enrichment of mosses and other plant species, which could result in higher organic carbon and nitrogen contents in the abandoned mounds than in primary mounds. As expected, the N-compounds decreased in the mounds when abandoned, which could be attributed to either alteration of the microbial communities or depletion of the nutrients in the aboveground part of the mound. Third, the secondary termites may rebuild the structure in the centre of the abandoned termite mound, which was determined by the significant differences in mean concentrations of nutrients found between young parts (locations 1–3, the values were similar to those of the primary mounds) and old parts (locations 4–6, the values approached those of the abandoned mounds) of the secondary mounds.

Furthermore, the data presented will be useful in better understanding the roles played by termites in nutrient cycling processes in tropical soils. Our study indicated that hot spots of nutrients occur that affect the dynamic equilibrium of nutrients at specific spatial and temporal scales through the fixation and redistribution of the nutrients. The spatial and temporal distributions of the properties in the mounds demonstrated an influence of termite activities on surrounding soils both laterally and vertically.

Acknowledgements

We thank Mr. Liu MN, Miss Fu Y, Miss Liu JQ, Miss Chen QF and the Central Laboratory of XTBG for their help. This research was supported by the National Natural Science Foundation of China (31570622 and 41701029), the Natural Science Foundation of Yunnan Province (2014HB042), the CAS 135-Program (No. 2017XTBG-F01), and the

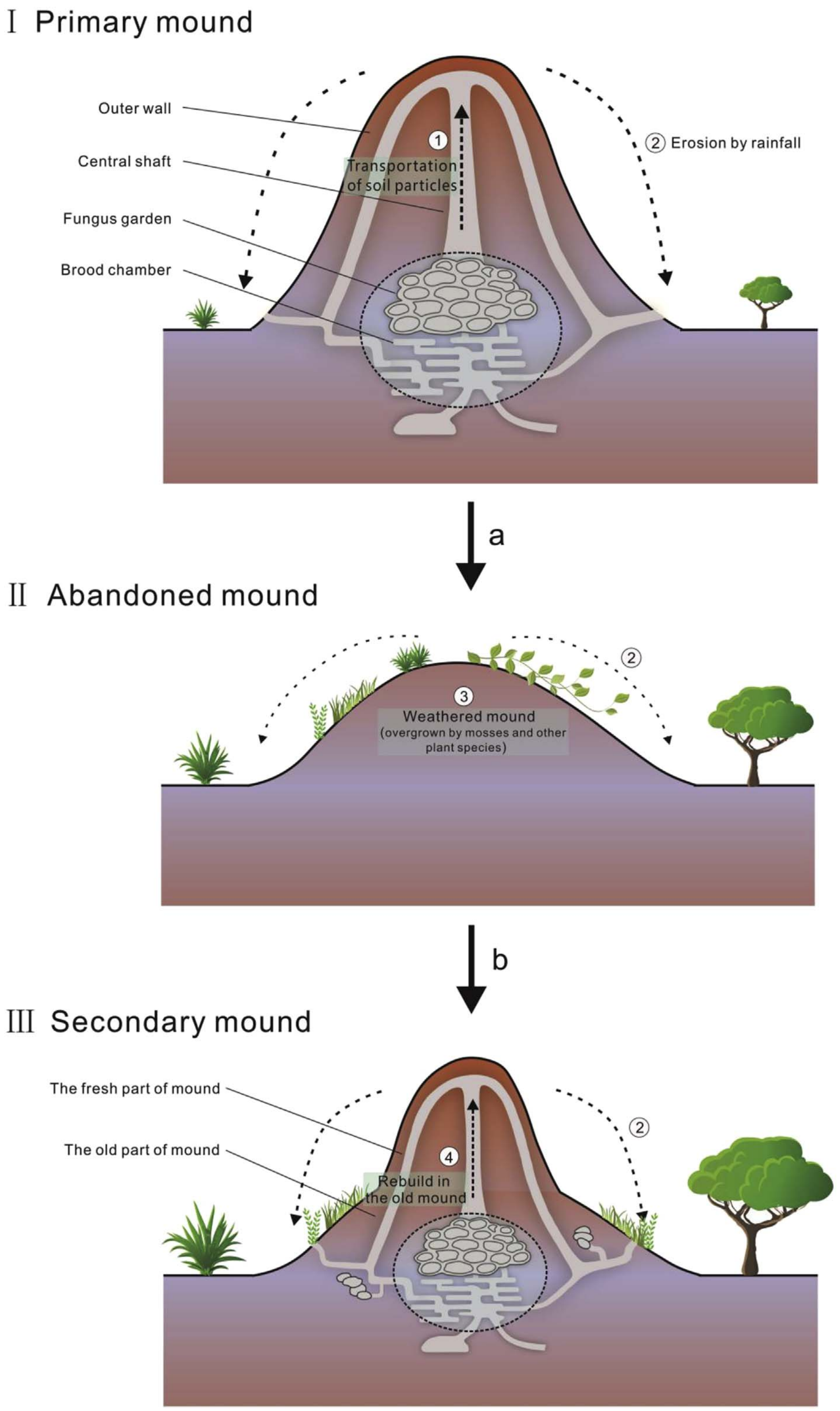

Fig. 9. Schematic diagram illustrating the relationship between the chronological development of termite mounds (primary, abandoned and secondary-occupied mounds) and the nutrient dynamics of the mounds. The termites build the primary mound (I) using a combination of saliva or faeces with soil particles, which are transported from deep soil layers (1; soil particle transportation), which results in more N-compounds and lower C_{org} and N contents in primary mounds than in adjacent soils. However, the mound may be abandoned (a) for various reasons, followed by soil erosion (2) and the invasion of plants (3) on the mound, which result in decreases in N-compounds and increases in C_{org} and N contents. Subsequently, the mound may be rebuilt by the secondary-occupied termites in the centre of the abandoned mound (b), which contributes to the nutrients in the fresh part of the mound approaching those in the primary mounds, whereas the nutrients in the old part of the mound approached the values found in the abandoned mounds.

Initiative Postdocs Supporting Program (BX201700278).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.11.018.

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