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Nitrogen mineralization, ammonia accumulation, and emission of gaseous NH_3 by soil-feeding termites

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Abstract. There are numerous reports on the accumulation of ammonia in the mounds of soil-feeding termites. Here, we provided direct evidence for an effective mineralization of nitrogenous soil organic matter in the gut of *Cubitermes* spp., which gives rise to enormous ammonia concentrations in the intestinal tract. In *Cubitermes ugandensis*, the ammonia content of the nest material [24.5 μmol (g dry wt.)$^{-1}$] was about 300-fold higher than that of the parent soil. Large amounts of ammonia were present throughout the intestinal tract, with lowest values in the extremely alkaline gut sections (pH >12) and highest values posterior hindgut [185 μmol (g dry wt.)$^{-1}$]. Results obtained with other *Cubitermes* species were similar. Ammonia concentrations in the posterior hindgut of these humivorous species (up to 130 mM) are among the highest values ever reported for soil macroinvertebrates and are matched only by insects feeding on an extremely protein-rich diet (e.g., the sarcophageous larvae of blowflies). Volatilization of ammonia [about 10 nmol (g fresh wt.)$^{-1}$ h^{-1}], either directly by emission from the termite body or indirectly from their feces, led to NH_3 concentrations in the nest atmosphere of *C. ugandensis* that were three orders of magnitude above the ambient background – a relative accumulation that is considerably higher than that observed with CH_4 and CO_2. Together with previous results, these observations document that through their feeding activity and due to the physicochemical and biochemical properties of their digestive system, soil-feeding termites effectively catalyze the transformation of refractory soil organic nitrogen to a plant-available form that is protected from leaching by adsorption to the nest soil. Nitrogen mineralization rates of soil-feeding termites may surpass those effected by tropical earthworms and should contribute significantly to nitrogen fluxes in tropical ecosystems.

Introduction

Termites are the most abundant insects in many tropical regions and may constitute up to 95% of all insect biomass in soil in certain habitats. They have a strong impact on soil fertility and are considered to play a keystone role in the biogeochemical cycles in their ecosystems (Wood 1988; Collins 1989; Lavelle et al. 1994, 1997; Wood 1996; Lopez-Hernandez 2001; and references therein).

More than half of the known termite genera are soil-feeders (Noirot 1992). Soil-feeding termites strongly influence many soil properties (for references, see

Brauman 2000; Brauman et al. 2000; Eggleton and Tayasu 2001), including the structural stability of soil organic matter (SOM) (Garnier-Sillam and Harry 1995; Mora et al. 2003), affect the distribution of organic matter and bacterial community structure in different particle size fractions (Fall et al. 2001; 2004), and are considered potentially important sources of microhabitat heterogeneity in tropical forest soils (Donovan et al. 2001b).

The most intriguing group among soil-feeding termites is the true soil-feeders (feeding group IV; Donovan et al. 2001a), which ingest mineral soil and are able to thrive on the most recalcitrant soil components. They comprise the *Cubitermes* branch of the Termitinae, an important component of the soil macrofauna in African tropical forests and wet savannahs (Eggleton and Tayasu 2001). Estimations of the annual soil consumption by *Cubitermes* species range from 1.2 to 4.5 kg m^{-2} (Wood 1978*; Lavelle et al. 1997), but little is known about their exact source of nutrition. Radiotracer studies have documented the capacity of *Cubitermes* species to mineralize a wide range of organic polymers, both in their free and humic-acid-stabilized form (Ji et al. 2000; Kappler et al. 2000; Ji and Brune 2001).

In contrast to sound wood, soil organic matter is rich in nitrogen. Most of the nitrogen in humic substances is present in amide or peptide structures (Schnitzer 1985; Knicker and Lüdemann 1995; Knicker et al. 1997; Vairavamurthy and Wang 2002). There is strong evidence that soil-feeding Termitinae are able to mobilize and digest the peptidic components of soil organic matter (Ji et al. 2000; Ji and Brune 2001), and that the extreme gut alkalinity in the anterior hindgut (up to pH 12; Brune and Kühl 1996) and alkali-stable and humic-acid-tolerant proteinases (Ji and Brune 2005) play a key role in this process.

So far, little is known about the role of the dense intestinal microbiota colonizing the different gut compartments of *Cubitermes* species (Schmitt-Wagner et al. 2003a, b). Nevertheless, it can be predicted that the mineralization of peptides and amino acids by the combined activities of the gut bacteria and host should eventually lead to the production of large quantities of ammonia. The latter would be released as NH_4^+ together with the feces, which are deposited on the inner wall of the nest, and – after deprotonization in the alkaline gut regions – also as gaseous NH_3 via the tracheal system.

Although soil-feeding termites are extremely abundant in tropical ecosystems, their contribution to the nitrogen cycle is only poorly investigated. Due to their enormous soil turnover, ammonia formation from humus may lead to a loss of bound nitrogen from soil, and the emission of gaseous NH_3 may even have implications for the atmospheric chemistry. To gain insight into these important matters, we determined the ammonia content in the soil around the nest, the nest material, and in the intestinal tract of several soil-feeding *Cubitermes* species. In addition, we measured the concentration of gaseous NH_3 in the internal nest atmosphere and its emission rates by the animals. In

* The original estimation by Wood (11.3 kg m^{-2}) apparently contains a decimal error: 2.76 g (g termite)$^{-1}$ d^{-1} × 365 d × 1.15 g termite m^{-2} = 1.16 kg m^{-2}.

accordance with the literature (Wright 1995), the term ammonia will be used for total ammonia, whereas NH_3 and NH_4^+ will refer to non-ionic ammonia or ammonium ions.

Materials and methods

Termites and soils

Soil-feeding termites (*Cubitermes* and *Procubitermes* spp.) were collected in various locations in Kenya, Senegal, and Congo (Brazzaville) (see Table 1), and were brought to the laboratory in whole nests or nest fragments as described elsewhere (Schmitt-Wagner et al. 2003b). Unless indicated specifically, all laboratory experiments were carried out within one month after collection. In all experiments, only worker caste termites were used.

Topsoil (0–5 cm depth) was collected directly around the nests and at about 3-m distance from the nests, where no activity of soil-feeding termites was observed. The pH of the soil samples ranged between 3.7 and 3.8, and that of the nest material between 4.1 and 4.4 (in 10 mM $CaCl_2$; determined with sample series I and II, see Table 1).

Extraction of ammonia and nitrate

Using fine-tipped forceps, the guts of 15 termites were dissected and separated into four sections, representing the major gut compartments (Figure 1). Gut sections were pooled in 1 ml of ice-cold 10 mM HCl, homogenized using an ultrasonic microprobe (10 W for 10 s), and incubated at 30 °C for 1 h with gentle shaking. Homogenates were centrifugated ($16{,}000 \times g$, 10 min), and the supernatants were analyzed for NH_4^+ and NO_3^-.

For the extraction of NH_4^+ and NO_3^- from nest material and soils, plant residues were removed from the samples, which were then dried and sieved to a particle size <2 mm. Aliquots of 1 g were extracted with 4 ml of 10 mM HCl or 12.5 mM $CaCl_2$ solution at 30 °C for 1 h, centrifuged, and supernatants were analyzed for total or exchangeable NH_4^+ and NO_3^-, respectively.

Sampling of gases from the nests

Gas samples were taken in the field, using stainless-steel tubes (30 cm length × 2 mm diameter) that were driven into the termite mound at different positions with a hammer. A solid stainless-steel rod inserted into the tube served to reinforce the tube and to prevent blockage of the tube by nest material. Immediately after removal of the rod, a 20-ml syringe was connected to the tube via a 3-way valve and gas samples were drawn at a rate of approximately 1 ml s^{-1}. Gas samples for CO_2 and CH_4 were transferred into two

Table 1. Ammonia and nitrate content in parent soil, nest material, and in different gut sections of soil-feeding termites.

Metabolite	Sample series[a]	Content [µmol (g dry wt.)$^{-1}$][b]						
		Soil		Nest material	Gut section			
		In 3-m distance	Around nest		M/ms	P1	P3	P4/5
Ammonia	I	0.085 ± 0.03	0.16 ± 0.08	24.5 ± 0.5	41.3 ± 2.6	7.2 ± 0.3	27.5 ± 3.0	185 ± 33
	II	0.012 ± 0.01	0.04 ± 0.02	14.0 ± 8.3	37.9 ± 2.1	15.7 ± 0.8	20.9 ± 2.4	177 ± 23
	III	n.d.[c]	0.19 ± 0.02	43.0 ± 17	57.0 ± 12	7.8 ± 0.8	27.3 ± 4.0	159 ± 44
	IV	n.d.	0.14 ± 0.02	10.6 ± 2.8	10.4 ± 1.5	4.1 ± 0.2	10.6 ± 2.3	103 ± 8.5
	V	n.d.	n.d.	18.9 ± 1.6	15.0 ± 3.0	3.4 ± 1.6	9.0 ± 0.8	77 ± 20
	VI	n.d.	n.d.	58.8 ± 2.6	17.2 ± 5.7	13.2 ± 6.3	30.4 ± 2.4	284 ± 36
Nitrate	I	0.18 ± 0.07	1.23 ± 0.17	0.72 ± 0.01	< 3	< 1	< 1	7.9 ± 0.6
	II	0.13 ± 0.01	0.29 ± 0.01	0.30 ± 0.03	< 3	< 1	< 1	8.2 ± 3.4
	VII[d]	n.d.	n.d.	8.0	12.0	2.2	8.6	10.3
	VIII[d]	n.d.	n.d.	5.5	6.1	< 1	1.8	4.8

Unless indicated otherwise, the values are means ± SD of three separate extractions.

[a]Mounds sampled: (I) *Cubitermes ugandensis*, open grassland, Kalunya Glade, Kakamega Rain Forest, Kenya; (II) *C. ugandensis*, steep grassland slope, Lirhanda Hill, Kakamega Rain Forest, Kenya; (III) *Cubitermes umbratus*, forest location, Shimba Hills Natural Reserve, Kenya; (IV, VII) *Cubitermes orthognathus*, savannah near Busia, Kenya; (V, VI) *Cubitermes* sp., grassland, Eastern Casamance Savannah, Senegal; (VIII) *Procubitermes* sp., forest location, Mayombe Rain Forest, Congo.

[b]Samples were extracted with HCl. When soils and nest material were extracted with $CaCl_2$, results did not differ significantly (data not shown).

[c]Not determined.

[d]Determined 2 months after collection (single measurements).

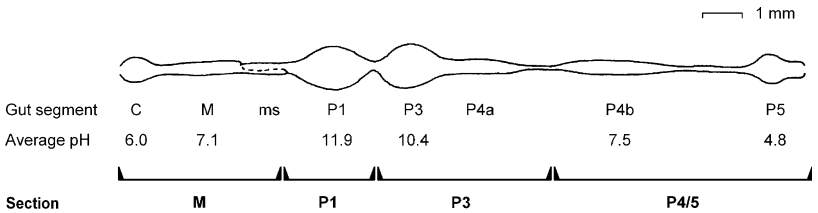

Figure 1. Gut morphology of a *Cubitermes* sp. worker termite. Intestinal tracts were dissected and separated into the indicated sections: M (crop, midgut, and mixed segment); P1 (proctodeal segment 1); P3 (proctodeal segment 3); P4/5 (proctodeal segments P4 and P5). The average luminal pH was determined for the gut segments of *Cubitermes speciosus*, using intact guts and glass pH microelectrodes (Brune and Kühl 1996).

butyl-rubber-stoppered 4.5-ml glass vials connected in-series to the sampling train via the 3-way valve; the process was repeated to ensure that the original atmosphere in the vials was completely replaced by the gas samples. Samples where analyzed for CO_2 and CH_4 within 1 or 2 weeks after collection, respectively; control experiments indicated that the recovery of CH_4 (100 ppmv) was 94 ± 6% ($n = 3$) after 21 days of storage.

For NH_3 measurements, 20 ml of the nest atmosphere was passed through an NH_3 trap, which was also connected to the sampling train via the 3-way valve, using the tandem sampling system of Karakas and Tuncel (1997) with slight modifications. The system consisted of a pre-filter and three sampling filters with a diameter of 25 mm. The pre-filter, a 1-μm pore size glass-fiber filter (GF 50, Schleicher & Schuell, Dassel, Germany), was used to remove dust and aerosols from the sample. Sampling filters were quantitative analytical filters (589/1, Schleicher & Schuell, Dassel, Germany) impregnated with oxalic acid. Before use, the analytical filters were washed with H_2O until the concentration of NH_4^+ in the rinse was < 1 μM, dried at 120 °C for 15 min, saturated with 0.1 M oxalic acid, and dried again. To prevent absorption of atmospheric NH_3, the filters were dried in a petri dish covered with a larger, but identically treated filter paper and stored in a tightly closed glass bottle. Directly before sampling, the sampling filters were re-wetted with 30 μl H_2O. The NH_4^+ collected on the filter was extracted and determined as described below. Controls were done in glass bottles (120 ml), containing 2 ml of known amounts of NH_4Cl to which 2 ml of NaOH (2 M) was added. Recovery rates were 99.8 ± 5.7% ($n = 3$); up to an NH_3 content of 1.5 μmol, 97 % of the NH_3 in the sample was collected on the first filter. The glass fiber filter did not absorb any ammonia during sampling.

NH_3 emission from living termites

About 260 freshly collected worker caste termites were placed into a 250-ml glass bottle (Schott) closed with a rubber stopper and equipped with an

ammonia trap, which consisted of a sampling filter (see above) suspended from the stopper. Filters were changed at definitive time intervals, and NH_3 content of the filters was determined. Bottles without termites were used as controls.

Analytical methods

The sampling filters were extracted with 1.8 ml deionized water, and NH_4^+ was analyzed using the colorimetric method of Bristow (1991) with modifications. Briefly, 0.4 ml extract was mixed with 0.3 ml reagent A (1.25 mM sodium nitroprusside, 0.4 M sodium salicylate) and 0.3 ml reagent B (4.5 mM sodium dichloroisocyanurate, 0.4 M NaOH) in 1-ml cuvettes. After incubation in the dark for 60 min, the absorbance at 657 nm was determined. NH_4^+ in the extracts of termite gut sections, soils, and nest material was determined by flow injection analysis (FIA) using the diffusion cell described by Hall and Aller (1992). The flow rate of carrier and receiving eluent (20 mM NaOH and 100 μM HCl) was 0.4 ml min^{-1}; NH_4^+ was quantitated using a conductivity detector.

NO_3^- was determined by high-performance liquid chromatography using an anion-exchange column (LCA A14, Sykam, Gilching, Germany) at 60 °C and a conductivity detector. The eluent consisted of 7.5 mM Na_2CO_3, 5% methanol (v/v), and 0.05% 4-hydroxybenzonitrile (w/v); the flow rate was 2.0 ml min^{-1}.

CH_4 and CO_2 were determined by gas chromatography on a molecular sieve column using a flame-ionization detector (Platen and Schink 1987). For CO_2, a methanizer was used to catalytically reduce CO_2 to CH_4 before detection.

Total carbon and nitrogen of soils and nest materials were analyzed with a CHN-Analyzer (Elementar Vario EL; Elementar Analysensysteme GmbH, Hanau, Germany).

Results

Ammonia and nitrate concentrations in soil, nest material, and gut sections

In all sample series, the ammonia and nitrate contents increased from the parent soil towards the nest (Table 1). Ammonia concentrations in the nest material itself (sampled from the inner nest) were up to three orders of magnitude higher than in the surrounding soil. The ammonia content of the individual gut sections differed strongly. In the midgut section, it was in the same range as in the nest material, decreased to a minimum in the alkaline P1 segment, and increased again in the posterior hindgut; highest values were found in the P4/5 section. The ammonia and nitrate contents in the gut sections of termites collected at different locations varied considerably, both between species of the same genus (*Cubitermes*) and even within the same species

(*C. ugandensis*). This might be due to differences in organic nitrogen content or quality of the material ingested by the termites in the respective locations.

The total amounts of organic carbon (TOC) in the soil samples was slightly lower than in the nest (Table 2), indicating an accumulation of organic matter in the nest material, which is largely constructed from feces (Eggleton and Tayasu 2001). The total nitrogen content of nest and soil samples did not differ significantly (Table 2), but the contribution of NH_4^+ to the total nitrogen in the nest material (up to 14%) was more than two orders of magnitude higher than in the parent soil (Table 3), documenting a strong mineralization of organic nitrogen during gut passage. Although nitrate was the major source of inorganic nitrogen in the soil samples (Table 3), gut contents and nest material always contained much more ammonia than nitrate (Table 1). Together with the strong increase of inorganic nitrogen in the gut, this rules out the possibility that ammonia is produced simply by microbial reduction of nitrate to ammonium (nitrate ammonification).

Figure 2 shows the absolute ammonia concentrations in the gut of *C. ugandensis*, calculated using the water content of the respective gut sections. Results were similar for all *Cubitermes* species investigated (see Table 1; details not shown). In all cases, the lowest values were found in the anterior, extremely alkaline hindgut section (P1). Highest ammonia concentrations were always present in the posterior hindgut section (P4/5), although there was no clear correlation between the ammonia contents of P4/5 section and nest material.

Emission of gaseous NH_3 by termites

When *C. ugandensis* worker termites, sampled from the nest about 30 h after collection, were placed into rubber-stoppered glass bottles, the NH_3 concentration in the headspace increased linearly with time (Figure 3). The average emission rate and a fresh weight of 7.2 mg per termite results in a specific NH_3 emission rate of 11.3 ± 0.9 nmol h^{-1} (g fresh wt.)$^{-1}$. Similar rates were

Table 2. Total organic carbon (TOC) and total nitrogen in parent soil and nest material of *Cubitermes ugandensis*.

Parameter	Sample series[a]	Content [mmol (g dry wt.)$^{-1}$][b]		
		Soil in 3-m distance	Soil around nest	Nest material
TOC	I	3.06 ± 0.29	3.28 ± 0.13	4.02 ± 0.06
	II	2.27 ± 0.00	2.38 ± 0.03	3.07 ± 0.19
Total N	I	0.21 ± 0.05	0.13 ± 0.02	0.19 ± 0.00
	II	0.08 ± 0.02	0.07 ± 0.01	0.10 ± 0.01
C/N	I	14.6	25.2	21.2
	II	28.4	34.0	30.7

[a]See Table 1 for details.
[b]Data are mean values ± deviation ($n = 2$).

Table 3. Ammonia and nitrate content relative to the total nitrogen content in parent soil and nest material.

Metabolite	Sample series[a]	Relative content (%)[b]		
		Soil in 3-m distance	Soil around nest	Nest material
NH_4^+	I	0.04 ± 0.02	0.12 ± 0.09	12.7 ± 0.3
	II	0.02 ± 0.02	0.06 ± 0.04	14.0 ± 9.5
NO_3^-	I	0.08 ± 0.05	0.96 ± 0.32	0.37 ± 0.01
	II	0.17 ± 0.05	0.43 ± 0.09	0.30 ± 0.06

[a]See Table 1 for details.
[b]Calculated from the data in Tables 1 and 2.

obtained with *C. umbratus* [9.6 ± 2.9 nmol h^{-1} (g fresh wt.)$^{-1}$] and *C. orthognathus* [8.3 ± 0.3 nmol h^{-1} (g fresh wt.)$^{-1}$] about two weeks after collection. Since the release of NH_3 from the feces did not differ significantly after the termites were removed from the vials (not shown), it was not possible to determine the proportion of NH_3 directly emitted by the animals.

Gas concentrations in the nest atmosphere

The internal atmosphere of the mounds of *C. ugandensis* was sampled on-site in Kakamega Forest, Kenya. The concentrations of NH_3, CH_4, and CO_2 were much higher than the atmospheric background at the sampling site (Table 4).

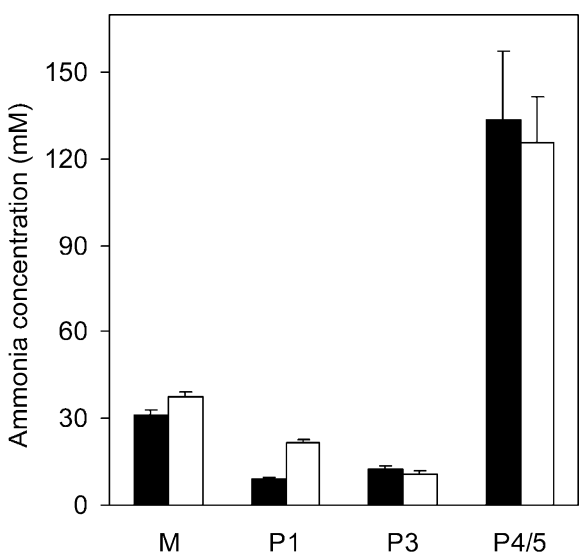

Figure 2. Average ammonia concentration in the gut of *Cubitermes ugandensis*, calculated from the absolute contents of ammonia (Table 1) and water (not shown) of the respective sections. Bars represent means and SD (n = 3) for sample series I and II.

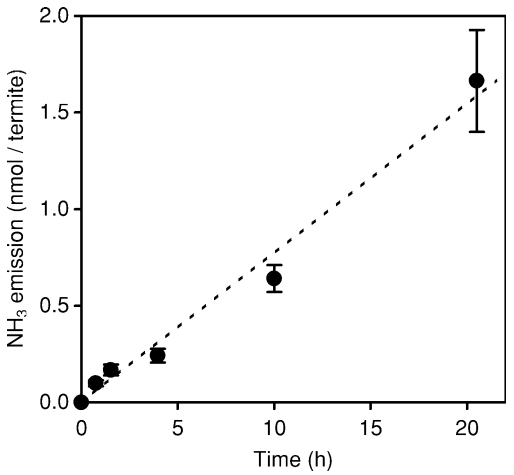

Figure 3. Time course of NH_3 emission from freshly collected *Cubitermes ugandensis*. Data points are means (±SD) for three replicates with termites from one colony (Kalunya Glade, Kakamega Forest); the regression line represents the average emission rate of 81 ± 6 pmol h^{-1} $termite^{-1}$.

On average, the observed accumulation rates were about 1600-fold, 75-fold, and 30-fold above atmospheric background for NH_3, CH_4, and CO_2, respectively. In one case, the NH_3 concentration in the nest surpassed the atmospheric value by a factor of almost 3000.

Discussion

There is a large body of literature on the accumulation of nutrients (C, N, P and available cations) in termite mounds and surrounding soils (Lavelle et al. 1994, 1997; Garnier-Sillam and Harry 1995; Lopez-Hernandez 2001; Ndiaye et al. 2004; and references therein). Here, we provide direct evidence for an effective mineralization of nitrogenous SOM in the gut of soil-feeding *Cubitermes* spp., which gives rise to an enormous accumulation of ammonia in the intestinal tract. Ammonia concentrations in the posterior hindgut of these

Table 4. Gas concentrations within different nests of *Cubitermes ugandensis* and in ambient air (Kalunya Glade, Kakamega Forest).

Gas	Internal atmosphere (ppmv)				Ambient air[a] (ppmv)
	Average ± SD	Max	Min	*n*	
CO_2	7600 ± 3000	13,100	4300	11	250 ± 60
CH_4	150 ± 110	300	23	11	2.0 ± 0.2
NH_3	19 ± 15	35	6	3	0.012 ± 0.004

[a]Air samples were collected 1 m above the ground in 5-m distance from the nest.

humivorous species are among the highest values ever reported for insects and are matched only by insects feeding on an extremely nitrogen-rich diet, e.g., the sarcophageous larvae of blowflies (Lennox 1940; Prusch 1971) and cockroaches raised on high-protein diet (Mullins and Cochran 1972, 1976).

Mineralization of nitrogen within the gut

The feces of wood-feeding termites, which usually feed on a lignocellulosic diet with a high C/N ratio (75–250; Tayasu et al. 1997), do not contain detectable amounts of any nitrogenous waste products. All uric acid entering the hindgut via the Malpighian tubules is immediately mineralized and reassimilated by the intestinal microbiota, which is considered an important strategy for nitrogen conservation (Potrikus and Breznak 1981; Slaytor and Chappell 1994; Breznak 2000).

The enormous amounts of ammonia excreted by soil-feeding termites are surprising only at first sight. The humic diet has a much lower C/N ratio (9–14; Tayasu et al. 1997) than that of wood-feeding species. In addition to mineral and humus particles, the guts of soil-feeding termites also contain plant tissue fragments, fungal hyphae, and numerous microorganisms (Sleaford et al. 1996; Donovan et al. 2001a), indicating that both humified and non-humified material is available for digestion.

However, little is known about the nature of the dietary components that are actually exploited. Humic substances are intimately associated with biochemically defined materials such as carbohydrates, proteins, and mucopeptides (Parsons 1988), and an acid hydrolysis of SOM releases up to 50% of the nitrogen as amino acids (Stevenson 1994). Spectroscopic analyses corroborated that most of the nitrogen in humic substances is present in amide or peptide structures (Knicker and Lüdemann 1995; Knicker et al. 1997; Vairavamurthy and Wang 2002).

Based on unpublished data, it had been suggested that the feces of *C. ugandensis* are depleted in peptides (B. Griffiths and D.E. Bignell, reported in Bignell 1994). Studies with radiolabeled humic model compounds substantiated that the peptidic component of humic substances is effectively mobilized and degraded, whereas the aromatic component is not mineralized to any significant extent (Ji et al. 2000), and that also microbial biomass and the aminoglycans of bacterial and fungal cell walls are potential food sources for soil-feeding termites (Ji and Brune 2001). Essentially the same results have been obtained also with the humivorous larvae of *Pachnoda ephippiata* (Li and Brune 2005a, b).

Routes of ammonia formation

While it is obvious that high concentrations of ammonia in the gut of soil-feeding termites are derived from bound nitrogen in the diet, the exact site of mineralization and the route of ammonia formation are not clear. In view of

the high proteolytic activity (Ji and Brune 2005) and the dense microbial colonization (Bignell et al. 1983; Schmitt-Wagner et al. 2003a) of the midgut region, the high ammonia concentrations in the M/ms section may be a direct consequence of microbial metabolism of the products of peptide and aminoglycan hydrolysis (i.e., amino acids and amino sugars). However, it is also possible that the monomers are resorbed by the midgut epithelium and metabolized by the insect.

Although most insects excrete the bulk of their nitrogen in the form of uric acid, a number of aquatic and terrestrial insect larvae return nitrogen to their environment in the form of ammonia (for references, see Wright 1995). In the cockroach *Periplaneta americana*, ammonia is released almost exclusively via the feces [90–170 nmol (g fresh wt.)$^{-1}$ h^{-1}], whereas the emission of NH_3 from the respiratory surfaces is negligible (Mullins 1974). At the alkaline pH encountered in the anterior hindgut segments (Figure 1), however, any ammonia formed in the gut lumen will be immediately deprotonized to NH_3, which would diffuse across the gut epithelium and, at least in part, be trapped in the neutral hemolymph as NH_4^+.

Ammonia is relatively toxic for most animals (for references, see Wright 1995), and the extremely high ammonium concentrations in the posterior hindgut of soil-feeding *Cubitermes* spp. (Figure 2) can be explained by a secretion of NH_4^+ into the P4 and/or P5 segment, which would be facilitated by the neutral to slightly acidic pH of these segments (Brune and Kühl 1996), which would act as an ''acid trap''. A similar mechanism has been suggested for Gypsy Moth larvae feeding on nitrogen-rich foliage (Lovett et al. 1998); here, the direct volatilization of ammonia was very low despite a midgut alkalinity that was as extreme as that of the P1 region of *Cubitermes* spp. (Brune and Kühl 1996). In the flesh-eating larvae of blowflies (*Sarcophaga* spp.), NH_4^+ is secreted directly into the hindgut by deaminating the amino acids in the hindgut epithelium, which avoids toxic ammonia concentrations in the hemolymph (Prusch 1971).

In soil-feeding termites, the continued emission of NH_3 after removal of the insects from the experimental setup indicated that the feces are the more important source of the NH_3 found in the nest atmosphere. Nevertheless, it is not possible to exclude that a small amount of NH_3 escapes via the tracheal system, since the animals mixed their freshly deposited feces immediately with their substratum.

Impact of ammonia on gut and nest processes

The high ammonia concentrations in the gut and the nest material of soil-feeding termites should have a fundamental impact on microbial activities in these habitats, and may also explain the relative low rates of microbial respiration reported for the nest material of *Cubitermes niokoloensis* (Ndiaye et al. 2004). A strong inhibition of methane oxidation by NH_3, as reported for

sediments and soils (Bosse et al. 1993; Schnell and King 1994), would explain the apparent absence of methanotrophic bacteria from the gut – a habitat that is characterized by high methane fluxes across the oxic-anoxic interface (Schmitt-Wagner and Brune 1999). Interestingly, stable-carbon-isotope fractionation indicates that up to 83% of the CH_4 produced within the nest of soil-feeding termites is still oxidized by the mound wall (Sugimoto et al. 1998) – despite the high ammonium concentrations in the nest material. It remains to be investigated whether the high concentration of ammonia in the mound may even stimulate CH_4 oxidation, as observed with rice field soils (Bodelier et al. 2000).

Nitrogen dynamics in nest and soil

The average ammonia content of the mound material of *Cubitermes* spp. amounted to 13–14% of its total nitrogen content (Table 3) and surpassed the inorganic N content of the parent soil by more than two orders of magnitude (Table 1), which corroborates that the bulk of the intestinal ammonia production is deposited with the feces. These findings are in agreement with other studies on soil-feeding termites *Nasutitermes ephratae* and *C. niokoloensis*, where ammonia represented up to 20 or 25% of the total nitrogen content of the termitaria, respectively (Lopez-Hernandez 2001; Ndiaye et al. 2004), suggesting that the strong mineralization of dietary nitrogen and the resulting accumulation of ammonia are general characteristics of all soil-feeding termites. Considering that ammonia can be stored by adsorption because of the cation-exchange ability of soil, while nitrate is easily leached out (Blackmer 2000), the cumulative evidence allows to conclude that soil-feeding termites effectively catalyze the transformation of refractory soil organic nitrogen to a plant-available form that is protected from leaching by adsorption to the nest material and the adjacent soil.

While the low nitrate contents of parent soil and nest material can be attributed to leaching or to uptake by plants, any nitrate consumed with the parent soil will be microbially reduced during gut passage. However, the reappearance of nitrate, with the highest contents in the posterior hindgut, indicates the presence also of nitrifying activities in the P4 or P5 segment (Table 1). It is possible that fecal nitrate alone is responsible for the elevated nitrate concentration in the mound material compared to the parent soil – despite the high availability of ammonia, potential nitrification rates in the mounds of *N. ephratae* and *C. niokoloensis* were found to be negligible (Lopez-Hernandez 2001; Ndiaye et al. 2004).

On the other hand, denitrification potentials in the mounds are reportedly higher than in the parent soil (Ndiaye et al. 2004), and also the observed enrichment of ^{15}N caused by soil-feeding termites (Tayasu et al. 1997) may be indicative of denitrification and ammonia volatilization, and – at least at high fluxes – also of nitrification (Nadelhoffer and Fry 1994). However, a

comparison of total nitrogen and C/N ratio in soil and nest material (Table 2) suggests that the feeding activity of soil-feeding termites does not cause a substantial loss of soil nitrogen, which may be explained by the protection of ammonia from leaching (see above).

Soil-feeding termites have an massive impact on the mineralization of nitrogenous soil organic matter. For *C. ugandensis*, the differences in inorganic nitrogen between parent soil and mound material are between 14 and 25 mmol per kg soil (Table 1, sample series I and II), indicating that 12 to 18% of the total nitrogen have been mineralized (Table 2). These values surpass considerably the net nitrogen mineralization rates given for the endogeic tropical earthworm *Pontoscolex corethrurus*, which were calculated from differences in ammonia content between ingested soil and casts, and ranged between 4 and 10% of total nitrogen for most of the soils tested (Lavelle and Spain 2001). Other estimates of the influence of earthworms on the net mineralization of nitrogen in bulk soil are generally lower (Edwards and Bohlen 1996), underlining the efficiency of nitrogen mineralization by soil-feeding termites. Based on the net mineralization rates for *C. ugandensis* (see above), the organic nitrogen content of the respective soils (Table 2, Sample series I and II), and an estimated soil processing rate by soil-feeding termites in a humid savannah of 4.5 kg m^{-2} a^{-1} (Lavelle et al. 1997), mineralization fluxes would range between 8.9 and 15.7 kg N ha^{-1} a^{-1}.

Due to their acidity, tropical rain forest soils should not cause any efflux of NH_3 into the atmosphere (Schlesinger and Hartley 1992). Although the enormous accumulation of ammonia in the mounds of soil-feeding termites give rise to elevated NH_3 concentrations in the internal atmosphere of the nest (Table 4), direct volatilization of NH_3 from termites or their feces is rather low. The average NH_3 emission rate of the three *Cubitermes* species investigated in this study is 9.7 nmol h^{-1} (g fresh wt.)$^{-1}$. Based on the estimated biomass of all soil-feeding termites in a humid savannah of 84 kg ha^{-1}, annual fluxes of NH_3 would not exceed 100 g N ha^{-1}, which would be of relevance only if the NH_3 accumulating in the internal nest atmosphere escapes from the mound into ambient air.

Conclusions

Together with the alkali-active and humic-acid-tolerant proteolytic activities present in the guts of these animals (Ji and Brune 2005), these findings substantiate our original proposal that microbial biomass and the nitrogenous components of humus, especially humic-acid-stabilized peptides, constitute an important dietary resource for soil-feeding termites and other humivorous soil macroinvertebrates (Ji et al. 2000; Ji and Brune 2001). In view of the abundance of soil-feeding termites in tropical ecosystems, their feeding activity will also dramatically affect the amount of nitrogen made available for plant growth and should contribute significantly to nitrogen fluxes in tropical soils.

The importance of direct volatilization of NH_3 from termites or their feces is negligible, although the significance for atmospheric fluxes remains to be clarified. Moreover, the high concentrations of CO_2, CH_4, and ammonia in the nest material make the mounds of soil-feeding termites an interesting model to study possible interactions between ammonia and methane oxidation.

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