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Influence of gut alkalinity and oxygen status on mobilization and size-class distribution of humic acids in the hindgut of soil-feeding termites

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Abstract

The majority of termite species do not feed on wood, but have a humivorous mode of nutrition. However, the exact nature of the carbon and energy source of soil-feeding termites, and also the role of the extreme alkalinity in their anterior hindguts are still unknown. Using soil-feeding *Cubitermes* species and high-pressure gel-permeation chromatography, we found a significant shift toward lower molecular weight in the humic acid samples extracted from different hindgut regions, compared to the parent soil ingested by the termites. The changes in size-class distribution were most pronounced in the highly alkaline (pH 12) P1 compartment, where the apparent molecular weight calculated for the peak maximum of the most abundant medium-molecular-weight fraction decreased by ≈30%. Microelectrode profiles demonstrated steep oxygen gradients into the alkaline hindgut compartments, caused by intestinal oxygen consumption in the gut periphery. In the less alkaline P3 region, oxygen consumption was at least partially attributable to biological processes, whereas in the highly alkaline P1 compartment, it seemed to be largely due to a chemical process. In vitro extraction of parent soil with NaOH solutions of increasing concentration confirmed that extraction efficiency of humic acids was not only enhanced by a high pH, but also by the simultaneous presence of oxygen. Similar results were obtained with 200 mM potassium carbonate solution, which mimics the pH and potassium concentration in the P1 region. However, in these experiments the pronounced molecular-weight shift found in the alkaline hindgut compartments was never observed, which indicates that additional factors must be present within the gut. We conclude that the extreme alkalinity in the anterior hindgut, supported by autoxidative processes, facilitates not only desorption of humic substances from the mineral matrix, but also decreases their molecular weight and increases their solubility. This renders so far unknown constituents of the humic matter accessible to microbial degradation in the subsequent, less alkaline hindgut compartments.

Keywords: Soil fauna; Humivore; Isoptera; Humic substances; Alkaline pH; Autoxidation

1. Introduction

Termites have a keystone role in controlling carbon and nitrogen fluxes both, in semiarid and humid ecosystems such as savannas and tropical rain forests (see Wood and Johnson, 1986; Collins, 1989; Martius, 1994; Bignell et al., 1997). Also their potential impact on agriculture is receiving increasing attention (Black and Okwakol, 1997). Different species of termites consume wood and litter in different stages of decay and humification, and more than half of all termite genera are considered humivorous (Noirot, 1992;

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Bignell, 1994; Bignell et al., 1997). Therefore, the influences of termites on soils range from physical effects to changes in the chemical properties of soil organic matter (e.g. disturbance of soil profiles, changes in soil texture and structural stability, nature and distribution of soil organic matter, C/N ratios). [For reviews, see Wood, 1988; Lobry de Bruyn and Conacher, 1990; and Brussaard and Juma, 1996.]

The importance of the humivorous soil fauna for the degradation and stabilization of organic matter is generally acknowledged (Insam, 1996), yet extremely little is known about the processes occurring during gut passage. Soil-feeding termites consume large amounts of soil (Okwakol, 1980); a gut-content analysis showed a large proportion of mineral and humus particles, but also plant tissue fragments, fungal hyphae, and numerous microorganisms (Sleaford et al., 1996). However, the identity of the specific components used as carbon and energy sources, the mechanisms involved in their digestion, and the role of the termite gut microflora in this process are unknown (Bignell, 1994; Bignell et al., 1997). Notably, the role of the distinct gut compartmentalization and the function of the elevated pH in the anterior hindgut (Fig. 1) is still obscure. Gut alkalinity is not considered as an adaptation to soil feeding per se, since it occurs also in wood-feeding species (Bignell and Eggleton, 1995), yet the most extreme alkalinities (pH >12) were found in soil-feeding Termitinae (Brune and Kühl, 1996).

Recent microsensor studies with wood-feeding termites have shown that the anoxic status of the hindgut is maintained by rapid oxygen consumption, which is attributed to the respiratory activity of the gut microbiota (Brune et al., 1995; Brune, 1998). However, a previous study of the soil-feeding termite *Cubitermes severus* had attributed the 'oxygen deficit' in homogenates of the alkaline hindgut compartments at least partially to a chemical reaction (Bignell and Anderson, 1980). Therefore, the oxygen status of the alkaline hindgut regions of soil feeders, and especially the importance of autoxidative processes in oxygen removal, require further investigation.

In this study, we determined the oxygen status and the nature of the oxygen uptake rates in the alkaline compartments. In addition, we investigated the influence of alkaline pH and oxygen on the extraction efficiency of humic acids from parent soil and, using high-pressure gel permeation chromatography, followed the changes in size-class distribution of humic acids during gut passage. The results help to elucidate the function of hindgut compartmentalization in soil-feeding termites and the role of different physicochemical conditions within these compartments in gaining access to the nutritive components contained in organic matter stabilized in organo-mineral soil aggregates.

2. Materials and methods

2.1. Termites

Cubitermes umbratus Williams (collected in the Shimba Hills National Reserve, Kenya) and *C. orthognathus* Emerson (collected near Busia, Kenya) were identified by Julius Muli, National Museums of Kenya. *C. speciosus* Sjöstedt was collected in the Mayombe rainforest, Congo (Brazzaville). Nest fragments were transported to our laboratory in polypropylene containers, and experiments were performed

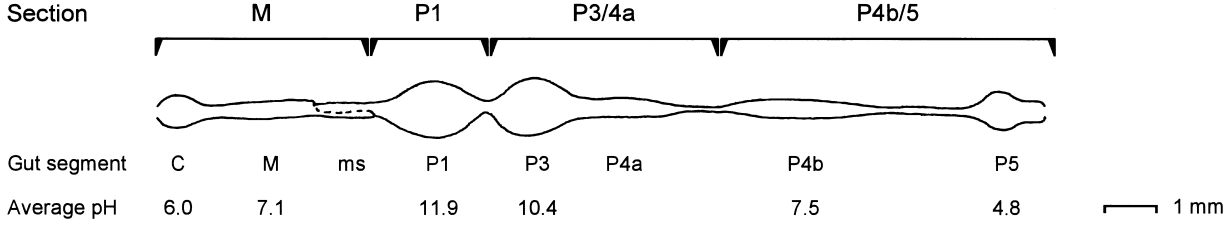

Fig. 1. Gut morphology of a *Cubitermes* sp. worker termite. For the extraction of humic acids, intestinal tracts were dissected and separated into the indicated sections (comprising the following gut segments): M (crop, midgut and mixed segment); P1 (first proctodeal segment); P3/4a (proctodeal segments P3 and P4a); P4b/5 (proctodeal segments P4b and P5). The average luminal pH was determined for the indicated gut segments in *C. speciosus* using intact guts and glass pH microelectrodes (Brune and Kühl, 1996).

within 2–4 weeks after collection. Although parent soil had been added to the containers, it has to be assumed that the termites were largely feeding on mound material during that period. Worker caste termites were used for all experiments.

2.2. Extraction of humic substances from soil samples

Topsoil (0–5 cm) was sampled at the collection site in Shimba Hills National Reserve from the vicinity of the nest (c.≈2–3 m distance). The soil was a Ferrosol with 2.0% organic carbon and a C/N ratio of ca. 20. Roots and small stones were removed, and the soil was mixed thoroughly. Humic substances were extracted using classical, solubility-based fractionation (Stevenson, 1994). Air-dried soil (5 g) was extracted with 20 ml 0.1 M NaOH at 30°C for 24 h in stainless steel centrifugation tubes under an N_2 atmosphere on a rotary shaker. The extract was separated from the insoluble residue (humins and inorganic matter) by centrifugation (13 000 × *g*, 30 min). Anoxic conditions were maintained during the entire procedure. After acidification with 1 M HCl to pH 1, the humic acids (HA) were allowed to precipitate for 24 h at 4°C, separated from the fulvic acids (FA) by centrifugation, and freeze-dried. All samples were stored at 4°C until analyzed.

2.3. Extraction of humic substances from termite guts

Termites were dissected in an anoxic glove box (N_2/3–5% H_2), and the guts were separated into four sections with fine-tipped forceps (Fig. 1). Gut sections were pooled (five each) in 0.5 ml 0.1 M NaOH in glass vials sealed by rubber-lined screw-caps, homogenized in an ultrasonic water bath for 5 min, and extracted for 24 h at 30°C under N_2 on a rotary shaker. After centrifugation (see above), the supernatant was acidified to pH 1 with 1 M HCl, and the humic acids were allowed to precipitate. After 24 h at 4°C, the fulvic acid fraction was separated from the humic acids by centrifugation.

2.4. High pressure gel permeation chromatography (HP-GPC)

The humic acid samples were dissolved in anoxic 0.1 M NaOH (1 mg/ml). Samples were filtered (cellulose acetate, 0.22 μm), and 50-μl aliquots were injected onto an HP-GPC column (length, 25 cm; diameter, 8 mm) filled with TSK HW 55 S gel (particle diameter, 20–40 μm; pore size, 300 Å, Grom, Herrenberg, Germany). The HPLC system was equipped with a UV detector and an autosampler. The mobile phase was 10 mM sodium phosphate buffer pH 11 (0.1 ml/min); the detection wavelength was 254 nm. To avoid autoxidation of samples, the eluent was carefully degassed and kept under N_2 during the measurements.

The column was calibrated with polyethylene glycol standards and showed a log-linear correlation between molecular weight and elution volume over a molecular mass range from 200 to 300 000 D. To minimize adsorption of humic acids to the hydrophobic column material, we increased the eluent pH from 7 to 11, which improved recovery of injected humic acids from <60% to >90%. The molecular weight distribution of humic acid samples was found to be largely concentration-independent between 0.05 and 2 mg/ml.

2.5. Radial oxygen profiles of hindgut compartments

Clark-type oxygen microelectrodes with guard cathodes (Revsbech, 1989) were constructed in our laboratory and calibrated as described by Brune et al. (1995). The microelectrodes had tip diameters of 10–15 μm. For the measurements, termite guts were dissected, unraveled, and embedded flat and fully extended within a glass microchamber in 1% agarose made in Ringer's solution (Ebert and Brune, 1997). The microelectrodes were positioned with a manual micromanipulator (MM33; Märzhäuser, Wetzlar, Germany). The minimum step increment was 50 μm; progress of the tip was observed with a horizontally mounted stereomicroscope. Usually, the fine microelectrode tip caused only a small deformation (20–50 μm) of the gut wall before penetration. This could not be avoided, and since the deformed gut wall rebounded after it was punctured with the electrode, only negligible bias of the actual position of the electrode tip relative to the gut wall was created. All measurements were performed at room temperature under air.

2.6. Oxygen uptake of termite gut sections

In an anoxic glovebox, 25 guts were dissected and the individual gut sections (Fig. 1) were pooled in 1 ml 0.2 M potassium carbonate buffer at in situ pH (simulating the high concentrations of carbonate ions in the hindgut fluid; A. Tholen and A. Brune, in preparation) and homogenized by brief sonication. Gut samples were kept on ice throughout the procedure. Aliquots (0.5 ml) of the homogenates were transferred into the glass cuvette of a Clark-type oxygen electrode (Rank, Cambridge, UK), which contained no gas headspace, and were mixed rapidly with a magnetic stirrer bar. Small volumes of freshly aerated buffer were added, and the consumption rate of dissolved oxygen recorded. Measurements were performed at 30°C.

3. Results

3.1. Effects of gut passage on humic acids

Humic acids were extracted from the parent soil, from the different gut sections depicted in Fig. 1, and from the nest material, which consists mainly of feces. Fig. 2 shows two typical HP-GPC chromatograms of humic acids prepared from the parent soil and from the P1 compartment, the most alkaline region of the anterior hindgut, illustrating the effects of the gut passage on the size-class distribution:

(i) a relative decrease in the abundance of molecules with high molecular weight (HMW);
(ii) a relative increase of those with low molecular weight (LMW); and
(iii) a significant shift toward smaller size among the molecules of medium molecular weight (MMW).

The absolute recovery of humic acids from the different gut sections were (in μg per gut section ±SD, $n = 3$): 47 ± 11 (M), 107 ± 31 (P1), 100 ± 10 (P3/4a), and 67 ± 12 (P4b/5). However, due to the differences both in gut volume and in density of the gut contents of each gut region, a comparison on a per-weight or per-volume basis was not possible. Therefore, we chose the relative abundances of the HMW and LMW fractions, calculated from the size-class boundaries defined in Fig. 2, and the shift in the apparent molecular weight of the

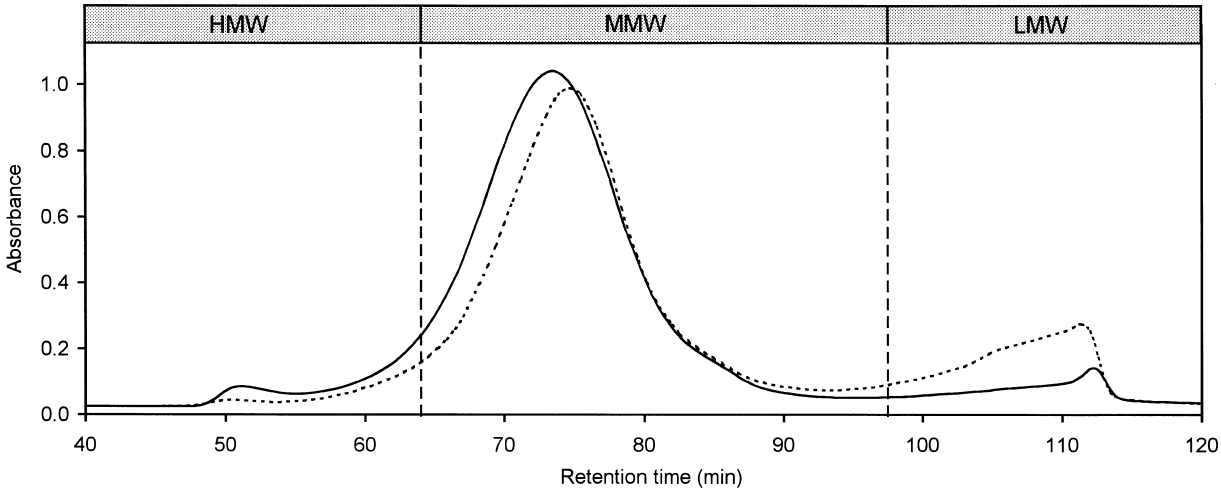

Fig. 2. Examples of typical gel permeation chromatograms of humic acids, illustrating the changes in size-class distribution between samples extracted from the parent soil (———) and the most alkaline anterior gut compartment (P1) of *C. umbratus* (- - -). Sample concentration was 1 mg/ml; absorbance was recorded at 254 nm. High-molecular-weight (HMW) and low-molecular-weight (LMW) fractions were arbitrarily defined by the retention times of polyethylene glycol standards ($M_r = 80\,000$ D and 3000 D, respectively), and were used as the basis for the quantitative comparisons reported in Fig. 3 and Table 1.

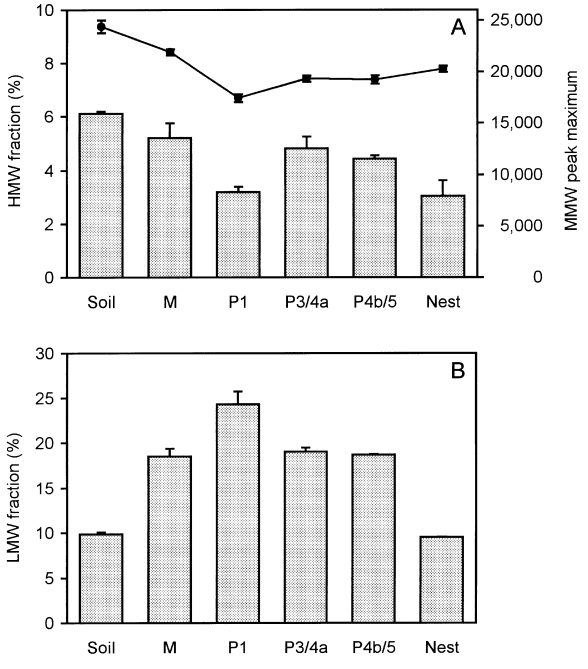

Fig. 3. Relative abundance of (A) high-molecular-weight (HMW) and (B) low-molecular-weight (LMW) fractions and (A) apparent molecular weight of the peak maximum of the medium-molecular-weight (MMW) fraction of humic acids extracted from parent soil, various gut sections (see Fig. 1), and nest material of *C. umbratus*. The calculations were based on the size-class boundaries indicated in Fig. 2; averages ± SD of three independent samples are given.

peak maximum of the MMW fraction for a quantitative description of the differences in size-class distribution between the samples.

Compared to the parent soil, the relative abundance of the HMW fraction was reduced in all gut regions, with an obvious minimum in the P1 compartment (Fig. 3(A)), whereas the corresponding LMW fraction (Fig. 3(B)) was significantly higher, with a distinct maximum in the P1 region. The majority of molecules were always of medium molecular weight, and showed a shift in the retention times of the peak maximum toward longer retention times for all hindgut sections, indicating a general reduction of their molecular weight (Fig. 3(A)). Again, this effect was most pronounced in the P1 region, where, compared to parent soil, a nominal reduction in the molecular weight of 29% was calculated (see also Fig. 2).

The results presented here were obtained with *C. umbratus*. However, the same shift toward smaller molecule size and similar effects of the gut passage on the relative abundance of the different size classes of humic acids were also observed with *C. orthognatus* (not shown).

3.2. Oxygen status of the alkaline hindgut compartments

Using microelectrodes, we determined radial profiles of the oxygen partial pressure around and within the alkaline compartments (P1 and P3) of *C. speciosus* hindguts embedded in agarose. In both cases, the oxygen concentration decreased linearly from the agarose surface toward the gut, identifying both hindgut dilations as significant oxygen sinks (Fig. 4). The slopes of the oxygen diffusion gradients were consistently steeper above the P3 compartment, indicating a flux of oxygen higher than that into the P1 compartment. However, the lower porosity in the P1 compartment, effected by its lower water content and evidenced by the sharper increase of gradient slope at the gut wall, apparently compensated for its lower oxygen consumption rate. As a consequence, the oxygen penetrating into the gut lumen was consumed completely within a distance of 100–150 μm below

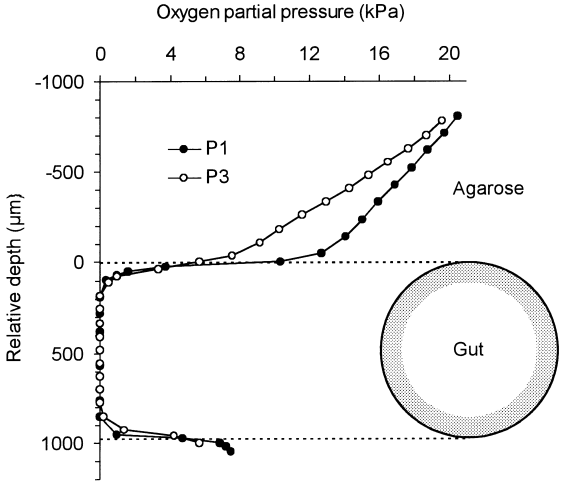

Fig. 4. Typical radial profiles of oxygen partial pressures above, and within, the agarose-embedded hindgut compartments P1 and P3 of *C. speciosus*, determined with microelectrodes. The dotted lines indicate the position of proximal and distal gut wall, the shaded area in the cross section illustrates the extent of oxygen penetration into the gut.

the gut wall in both compartments. Also the smaller hindgut compartments completely consumed oxygen within the gut periphery (not shown). A comprehensive study of oxygen and hydrogen gradients in the hindguts of *Cubitermes* spp. will be published elsewhere (D. Schmitt-Wagner and A. Brune, in preparation).

Gut homogenates of the P1 and of the P3/4a sections, prepared with anoxic buffer representing the average pH within the respective section, exhibited significant oxygen consumption rates when oxic buffer was added to the suspensions. Initial rates of oxygen uptake were in the range of 0.5–0.9 nmol min^{-1} gut^{-1} for the P1 and 0.2–0.8 nmol min^{-1} gut^{-1} for the P3/4a homogenates (pH 11.9 and pH 10.4, respectively), and rapidly declined with time. When preincubated with formaldehyde, the oxygen consumption rates of P3/P4a homogenates decreased to 0.1–0.5 nmol min^{-1} gut^{-1}; in each poisoned homogenate, the rate always being significantly lower than that of the untreated control, indicating the presence of a biological activity. In contrast, oxygen consumption of P1 homogenates remained unaffected by formaldehyde, indicating a purely chemical nature of the oxygen-consuming activities within the most alkaline gut region.

3.3. Influence of physicochemical parameters on extraction efficiency

The obvious changes in size-class distribution of humic substances in the alkaline hindgut compartments, especially in the P1, and the high oxygen uptake rates of these compartments prompted us to investigate the significance of the special physicochemical conditions characterizing these gut regions for the extraction of organic matter from the soil matrix. When we extracted parent soil with NaOH solutions of increasing concentrations under N_2, we found that the extraction efficiency increased with the NaOH concentration up to and including pH 13 and then decreased again with 1 M NaOH (Table 1), a phenomenon already described by Levesque and Schnitzer (1965). The amount of humic acids extracted with 200 mM potassium carbonate solution (pH 12), which resembles the pH and the major

Table 1
Effect of pH and O_2 on extraction efficiency of humic acids from parent soil with NaOH solutions of increasing alkalinity, and size-class distribution within the humic acids determined by HP-GPC

Solvent	pH	O_2	Humic acids extracted [a] (mg/g soil)	Increased extraction with O_2 (%)	Size-class distribution (mg/g soil) [b]		
					HMW	MMW	LMW
H_2O		− [c]	6.4 ± 0.3		0.4	5.1	0.9
		+ [d]	7.0 ± 0.3	9.4	0.4	5.2	1.4
NaOH 1 mM	11	− [c]	7.9 ± 0.7		n.d. [e]	n.d. [e]	n.d. [e]
		+ [d]	11.0 ± 0.9	39.2	n.d. [e]	n.d. [e]	n.d. [e]
NaOH 10 mM	12	− [c]	25.5 ± 0.4		n.d. [e]	n.d. [e]	n.d. [e]
		+ [d]	33.6 ± 0.8	31.8	n.d. [e]	n.d. [e]	n.d. [e]
NaOH 100 mM	13	− [c]	34.3 ± 0.8		3.9	28.3	2.1
		+ [d]	38.9 ± 0.8	13.4	4.6	32.2	2.1
NaOH 1 M	14	− [c]	24.0 ± 0.4		0.4	21.8	1.9
		+ [d]	25.6 ± 0.7	6.7	0.9	22.8	1.8
K_2CO_3 200 mM	12	− [c]	19.5 ± 0.3		1.1	17.3	1.1
		+ [d]	23.4 ± 0.3	20.0	1.0	21.2	1.3

[a] Averages ± SD ($n = 3$).
[b] Based on the size-class boundaries indicated in Fig. 2; SD omitted for clarity.
[c] N_2 headspace.
[d] Air headspace.
[e] Not determined.

electrolyte in the most alkaline P1 compartment, came close to that of the NaOH solution with the same pH.

Since the microsensor measurements indicated oxygen uptake by the alkaline gut compartments and the existence of an oxic periphery around the anoxic lumen, we repeated the extraction experiment in the presence of oxygen. While the general pH effect was similar to that obtained under N_2 atmosphere, the extraction yields of all alkaline solvents increased significantly when oxygen was present (Table 1). HP-GPC analysis showed that, in both cases, the increase in extraction efficiency was correlated with an increased abundance of large- and medium-sized molecules; the amount of molecules recovered in the LMW fraction remaining relatively constant (Table 1).

4. Discussion

The role of termites in the control of important physical and chemical soil parameters such as porosity, texture, nitrogen content, and the formation of soil aggregates and clay-mineral complexes is widely recognized (see Anderson, 1988; Wood, 1988; Garnier-Sillam and Harry, 1995; Garnier-Sillam and Toutain, 1995; Brussaard and Juma, 1996). There is, however, little information on the direct effects of gut passage on the ingested soil organic matter. In this paper, we showed for the first time that distinct changes in the size-class distribution of humic acids occur during the gut passage, especially in the anterior hindgut. We provided evidence that the dramatic molecular weight reduction in the humic acids is caused by (i) the extreme alkalinity in this gut region, which significantly increases the solubility of soil organic matter, and (ii) autoxidation of humic substances due to oxygen diffusing rapidly into the alkaline gut compartments.

4.1. Role of gut alkalinity

Although the extreme pH changes along the gut axis of many higher termites appear to be taxon- rather than diet-dependent, they are regarded as an important prerequisite for soil feeding (Bignell and Eggleton, 1995). The P1 compartment in the anterior hindgut of soil-feeding termites exhibits the most extreme alkalinity ever encountered in biological systems (Brune and Kühl, 1996). Similarly high pH values have been reported only for the midguts of certain dipteran, lepidopteran and coleopteran larvae (Bayon, 1980; Martin et al., 1980; Dow, 1984). It has been postulated that midgut alkalinity is an evolutionary adaptation to a diet rich in tannins or other polyphenolic constituents, since it enhances the solubility of dietary proteins and prevents precipitation of digestive enzymes (Berenbaum, 1980; Sharma et al., 1984; Martin et al., 1987; Felton and Duffey, 1991).

Soil-feeding termites, and possibly also humivorous coleopteran larvae ingest soil organic matter that is stabilized by an intimate association with inorganic soil constituents. The strategy for gaining access to this otherwise abundant dietary resource seems to be the sequestration of organic nutrients from the organo-mineral aggregates by alkaline extraction. The increased extraction efficiency of humic acids from soil at alkaline pH can be explained partly by an increased polarity, resulting from the deprotonation, e.g. of phenolic residues, and partly by the desorption of organic matter stabilized in clay-mineral complexes (Stevenson, 1994). Our results showed that the enhanced extraction efficiency of humic acids is based mainly on an increased recovery of molecules with medium and high molecular weights, whereas the amount of smaller molecules was largely pH-independent (Table 1). It is likely that the increase in extraction efficiency with increasing alkalinity is still underestimated since chemical hydrolysis of humic substances at high pH gives rise to products that are no longer recovered in the acid-precipitable humic acid fraction but rather in the acid-soluble fulvic acid fraction (Khairy and Ziechmann, 1981).

It has been reported that the alkaline hindgut regions of soil-feeding termites (P1-P3) show a significantly elevated potassium content (Bignell et al., 1983). Since these gut regions also contain high concentrations of carbonate ions (A. Tholen and A. Brune, in preparation), it is most likely that the alkalinity of the termite hindgut fluid is based on the same mechanism that generates the high pH in caterpillar midguts, namely an ATP-driven secretion of potassium carbonate (Dow, 1992). At a pH of 10.8, the potassium ion concentration in the midgut fluid of the tobacco hornworm is 200 mM (Dow and Harvey, 1988). Therefore, we chose a 200-mM K_2CO_3 solution to mimic the physicochemical conditions within

the alkaline P1 compartment of soil-feeding *Cubitermes* (pH 11.9 ± 0.3; Brune and Kühl, 1996), and found that the extraction efficiency of this solution came close to the value obtained with an NaOH solution of identical pH (Table 1).

4.2. Role of oxygen

It has been shown that the anoxic status of the enlarged hindgut compartments of wood-feeding termites is caused by rapid oxygen consumption by the gut microbiota (Brune et al., 1995), and also the alkaline gut regions of soil-feeding termites appear to be largely anoxic (Brune, 1998). However, while the moderately alkaline P3 compartment supports a dense bacterial microbiota, the number of microorganisms is much lower in the extremely alkaline P1 region (Bignell et al., 1980). Nevertheless, both compartments efficiently consume oxygen, and are rendered anoxic within the first 100 μm below the gut epithelium, as indicated by the oxygen profiles (Fig. 4).

Oxygen consumption by gut homogenates of the less alkaline P3/4a region decreased by ≈25% when homogenates were poisoned with formaldehyde, indicating the presence of a biological component, whereas in the case of the extremely alkaline P1, oxygen uptake rates seem to be based on purely chemical processes such as autoxidation. This agrees with an earlier study by Bignell and Anderson (1980), who found that a major part of the 'oxygen deficit' in gut homogenates of *C. severus* was not abolished by poisoning with cyanide and they, therefore, attributed it to a chemical reaction.

The rate of autoxidation should increase with gut alkalinity (Swift and Posner, 1972; Parsons, 1988), but the oxygen uptake rate of the P3 compartment is higher than that of the P1 (Fig. 4), which underlines the importance of biological oxygen consumption in the P3 compartment. In addition to the direct physiological effects of the alkaline pH, also the detrimental effects of phenolic radicals formed during autoxidation (Felton and Duffey, 1991; Appel, 1993) may explain the suppression of the microbiota in the P1 region.

Since the chemical reaction of O_2 with organic matter does not necessarily lead to the formation of stoichiometric amounts of CO_2, high rates of autoxidation would inevitably affect the insect's respiratory quotient (RQ), i.e. the ratio of CO_2 production to O_2 consumption. Therefore, autoxidation may serve to explain the extremely low RQ values reported by Rouland et al. (1993) for several soil-feeding Termitinae (0.36–0.67), although these values are questioned by the study of Nunes et al. (1997), who found the RQ of soil feeders to be always around or significantly above unity. More research is needed to resolve this issue.

Autoxidation of alkaline solutions of humic acids leads to molecular weight reduction, hydrolytic release of amino acids, and an increased content of polar carboxyl and keto groups (Dubach et al., 1964; Swift and Posner, 1972; Parsons, 1988; Kipton et al., 1992). This explains why the solubility of humic acids increased when soil was extracted with alkaline solvents in the presence of oxygen (Table 1), but the results of the chemical extraction alone do not fully account for the effects of gut passage on size-class distribution of humic acids. In particular, the strong molecular weight shift, which is most obvious when comparing the results for the highly alkaline P1 compartment to those for the parent soil (Fig. 3), was never observed among the samples obtained by simple extraction. This indicates that the effects of gut passage cannot simply be attributed to a combined action of alkaline and oxic conditions, but that other, so far unknown factors are present within the gut. Rouland et al. (1986, 1989) have reported the presence of several hydrolytic activities (polysaccharidases and other carbohydrases) in the midgut, hindgut and salivary glands of *Crenetermes albotarsalis* and *Thoracotermes macrothorax*. More work on other species of soil-feeding termites and other classes of enzymes is urgently required.

4.3. Determination of size-class distribution by HP-GPC

The hydrodynamic size of humic acids is strongly dependent on sample concentration, ionic strength, and pH value of the solution (Schnitzer, 1991). Therefore, and since calibration curves obtained with different molecular weight standards (dextrans, proteins, polystyrene sulfonates, and polyethylene glycols) differ significantly, the absolute molecular weights reported for humic acids have to be regarded with

caution (Goh and Reid, 1975; Chin et al., 1994; Piccolo et al., 1996). Additional bias is added by the fact that aromatic copolymers, as used in pressure-stable HP-GPC resins, show strong hydrophobic interactions with humic substances, especially when neutral or weakly alkaline eluents are used (Swift, 1985; Stevenson, 1994; Anderson and Hepburn, 1977). The alkaline mobile phase used in this study eliminated the strong interaction of humic substances with the hydrophobic column material (see Section 2, and also Abbt-Braun et al., 1991; Town and Powell, 1993), although slightly different retention times for monomeric substances of different polarity still indicated that non-polar interactions were not completely abolished (data not shown). However, as long as only relative changes of molecular weight distribution between different samples are being compared, HP-GPC of humic substances can be regarded as a useful method for following dynamic changes caused by transformation and degradation of humic substances during the gut passage.

5. Conclusions

In soil-feeding termites, alkaline gut regions serve to sequester organic nutrients from the ingested soil by increasing the solubility of humic substances, probably breaking up stable organo-mineral complexes. The effect of the alkaline extraction in the P1 is enhanced by chemical oxidation due to the oxygen supply via the gut epithelium, whereas in the next, slightly less alkaline P3/4a region, microbial activities become increasingly important. The degradation products formed in the posterior gut regions support the microbiota and, directly or indirectly via the fermentation products, also the host. Reprecipitation of the organic matter mobilized in the P1 by the neutral to acidic conditions in the posterior hindgut, and also microbial activities in the freshly voided feces may explain the observed differences between the posterior hindgut regions (P4b/5) and the nest material, which is constructed largely of fecal matter combined with parent soil (Wood, 1988). Such maturation and stabilization processes may be responsible for the increased structural stability reported for soil that was subjected to the activity of soil-feeding termites (Garnier-Sillam and Harry, 1995).

In future research, it will be important to identify the exact nature of the humic constituents which are mobilized and degraded, e.g. using specifically radiolabeled model substrates. An important aspect is the direct effect of gut passage on the composition of soil organic matter; while readily hydrolyzable components are removed, fresh microbial biomass is added to the humic substances voided with the feces. Also stabilizing effects caused by the reprecipitation of humic substances in the posterior hindgut, and the formation of new aggregates and organo-mineral complexes need to be elucidated.

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