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Application of ^{13}C NMR to investigate the transformations and biodegradation of organic materials by wood- and soil-feeding termites, and a coprophagous litter-dwelling dipteran larva

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Abstract

Solid-state ^{13}C nuclear magnetic resonance spectroscopy has been used to characterize the C in samples of the food (wood), gut contents and faeces from the wood-feeding termite, *Microcerotermes parvus*; soil in the guts and mound material from the soil-feeding termite, *Thoracotermes macrothorax*; and the food and faeces from the litter-feeding, coprophagous larvae of the dipteran fly, *Bibio marci*. Spectra from the wood-feeding termite indicated preferential loss of polysaccharide and accumulation of lignin with some modification to the O-aromatic-C and methoxyl-C (O-methyl-C) components during passage through the gut. Spectra for the soil-feeding termite indicated little change in the distribution of ^{13}C between resonances following passage through the gut, except for some evidence of preferential polysaccharide loss. Interpretation of the spectra from these organisms was restricted by the relatively low C content of the soils and mound material, and by the large contribution to the NMR spectra from the gut tissue rather than the gut contents. Spectra for the litter-feeding dipteran larvae indicated preferential feeding on the polysaccharide-rich component of the litter and then overall loss of polysaccharide-C and accumulation of both aromatic-C and methoxyl-C in the gut. These changes were greater for the second passage than for the first passage through the gut, suggesting that principally mechanical and physical changes occurred initially and that chemical digestion was prevalent during the second passage.

Introduction

Invertebrate animals representing many phyla are found in soils and associated with organic residues such as decomposing litter and wood. Whilst these organisms are not as abundant as the microbial decomposers, they nevertheless make an important contribution to the biodegradation of organic residues in natural and semi-natural environments. This is because in addition to their digestive mechanisms, they fragment residues, promote microbial activity and in some cases harbour specialised intestinal microbial communities that facilitate organic transformations. Of the larger and more conspicuous animals, the earthworms (Annelida, Oligochaeta) which contribute to biodegradation of natural residues primarily by altering physically the residues and the soil matrix, thereby enhancing microbial activity, have been most widely investigated (Coleman and Crossley 1996). This paper focuses on the roles of selected insects (Arthropoda) in biodegradation, which have been less well investigated and which in some cases contrast markedly with

those of earthworms. The insects we considered are two species of types of termite with contrasting life-styles: *Thoracotermes macrothorax*, is a soil-feeder that is permanently resident in soil with an epigeal soil-constructed mound; and *Microcerotermes parvus*, is a wood-feeder that is transiently-resident in soil (*sensu* Wallwork 1970), nesting in fallen wood (population ecology and other details of the termites can be found in Eggleton et al. (1996)); and the litter-feeding, coprophagous larvae of the dipteran fly, *Bibio marci*, which can be considered as temporary denizens of the soil (*sensu* Wallwork 1970).

Termites are important members of the soil community in tropical regions, where they are major determinants of soil structure, especially porosity and the stabilization of organic matter, thus regulating organic residue decomposition and soil fertility (Lee & Wood 1971; Garnier-Sillam & Harry 1995; Brussaard & Juma 1996). Termites of the more primitive families, such as the Kalotermitidae, possess an intestinal microbial community including protozoa, which facilitate the digestion of cellulose, and allows them to feed on wood. In some parts of the world, some such wood-feeding termites are important economic pests (Lee & Wood 1971). Termites of the more evolutionarily more advanced termites (Family Termitidae) have a complex intestinal microbial community including archaea, bacteria (including actinomycetes) and fungi, but not flagellate protozoa, and are able to feed (in different species) on wood and on humified organic matter in soils (Breznak & Brune 1994; Bignell 1994). Many other insects are temporary soil residents, i.e., they inhabit the soil or litter at particular stages in their life-cycle (Wallwork 1970). The larvae of the St. Mark's fly, *Bibio marci*, is a common inhabitant of soil in deciduous temperate forests which has such a strategy and can play important role in leaf litter decomposition (Karpachevsky et al. 1968; Szabo 1974). Besides feeding on leaf litter, they are also coprophagic (they consume their faeces), which enables them optimally to exploit the resource (Hassell & Ruston 1984; Gunnarson & Tunlid 1986).

One of the main restrictions to the investigation of the roles of soil invertebrates in the transformation and biodegradation of organic materials is the difficulty in chemically characterizing, at the level of broad classes of organic compounds, the substrate, the gut contents and faeces, all of which may be mixed with soil. Solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy has been widely used to characterize soil organic matter and decomposing plant material (Wilson 1987; Skjemstad et al. 1997; Hopkins & Chudek 1997), because it allows semi-quantitative determinations of a range of functional groups of C that are indicative of different classes of biochemicals (Table 1) to be made without the need to extract particular fractions prior to analysis. This technique has not been widely applied to characterize the transformations associated with invertebrates during the biodegradation of organic residues, except with earthworms: Guggenburger et al. (1996) used ^{13}C NMR amongst other techniques to show that the casts of anecic (those which move between mineral soil horizons and surface litter at the surface) earthworms in a tropical soil produced casts that were composed of relatively undecomposed plant litter than the mineral soil; and Vinceslas-Apka & Loquet (1997) used NMR to show the rapid preferential loss of polysaccharides in vermicomposted maple prunings.

In the present work we have used solid-state ^{13}C NMR to investigate the effects of passage through the guts of wood-feeding termites, soil-feeding termites and litter feeding, coprophagous dipteran larvae on the distribution ^{13}C between different functional groups in the food, the residual food (where appropriate), the gut contents and the excrements.

Materials and methods

Workers of *M. parvus*, samples of the wood in which they fed and nested, together with nest carton (composed largely of aged faecal material) were collected in the Mbalmayo Forest Reserve, Cameroon, West Africa during March 1995. The guts plus their contents were removed from several hundred *M. parvus* by dissection of fresh specimens.

Workers of *T. macrothorax* were collected and dissected at the same time as those of *M. parvus* and material from the guts including their pluscontents were divided in to separate crop and hindgut samples. Samples of the *T. macrothorax* mound, which is composed mainly of egested soil, were also collected.

Individuals of *B. marci* larvae were collected in a deciduous woodland dominated by *Carpinus betulis* (hornbeam trees) near Vranov nad Dyji Česke Budějovice in the Czech Republic. In a laboratory experiment, the larvae were supplied with either hornbeam leaf litter and or with their own 0–3 days old excrements as a food. Details of this experiment experimental conditions are published elsewhere (Frouz & Šustr 1996). Samples of the food, the residual food

Table 1. Chemical shift ranges, chemical assignments and classes of compounds (Wilson 1987)

Shift range (ppm)	Type of C	Main classes of compounds included
10–45	Methyl- & alkyl-C	Lipids, waxes and aliphatic hydrocarbons
45–60	Methoxyl- & N-amino-C	Lignin substituents, amino acids and amino sugars
60–90	O-alkyl-C	Carbohydrates
90–110	Acetal- & ketal-C	Carbohydrates
110–160	Aromatic-C	Phenyl-propylene sub-units of lignin
160–200	Carbonyl-C	Organic acids and amino acids/peptides

after feeding, and the faeces after both the first passage and second passages through the gut were collected.

All the materials were air-dried immediately after collection and ground with a mortar and pestle prior to NMR. The Fe concentrations of the *T. macrothorax* gut and mound materials were diminished by treatment with sodium dithionite and hydrochloric acid (Arshad et al. 1988). ^{13}C NMR spectra were collected under cross-polarization (CP), magic angle spinning (MAS) conditions using a Chemagnetics CMX LITE 300 MHz NMR spectrometer (^{1}H, 300.63 MHz; ^{13}C, 75.46 MHz) in zirconia rotors with Kel-F caps which contained between 0.4 and 0.7 g of the particular materials (depending on density) when packed. The NMR operating parameters were 1 ms contact time, 2 s relaxation delay, 4 μs 90° pulse and 4 kHz MAS. Spectra were recorded until optimal signal-to-noise ratios were obtained which ranged between 1500 and 16000 scans.

C and N concentrations in the samples were determined using a Carlo-Erba CHN analyzer.

Results and discussion

M. parvus wood-feeding termite

The C concentration was greatest for the wood (food) and least for the nest carton material (Table 2). This is consistent with a relative loss of C during passage of the food through the gut, although in the case of the nest carton material it may also reflect mixing with some low C material during nest building. The C concentration of the gut samples fell between those of the wood and the nest carton, but this material contained C from both the gut contents and the gut wall itself. The N concentration of the gut samples was greater than that of the other materials, both of which were close to zero (Table 2). It is likely that this was due primarily to the protein of the gut wall with possibly a contribution from microbial-N in the gut lumen.

The NMR spectra (Figure 1) differed markedly between the materials. In the alkyl-C region (10–45 ppm) there was a weak signal centred at about 15 ppm for the wood, a set of strong but broad signals with separate peaks at about 15, 20, 25 and 35 ppm for the gut material and virtually no signal in this region for the nest carton. The additional C in the alkyl-C region of the gut samples could be due to lipid components of the gut wall or metabolites in the gut, or both. If the enhanced signal in the alkyl-C region of the gut samples was due to alteration of their chemical characteristics, a corresponding increase in the alkyl-C for the nest carton would also be expected. The fact that no such increase was observed indicates that the alkyl-C in the gut sample was due to the gut tissue rather than the contents. Whilst microbial metabolites, such as acetate (ethanoate) may accumulate in termites' guts (Anklin-Muhlemann et al. 1996) prior to assimilation, this would not necessarily give a corresponding signal in the mound material. Acetate would give a methyl-C (close to 0 ppm) resonance. Although there were some signals at chemical shifts less than 20 ppm, it seems unlikely that acetate made a large contribution to these because there were too many subsidiary signals and the overall resonance was broad. Furthermore, acetate assimilation from the gut makes it unlikely that the acetate concentration in the carton material would be great enough to account for the strong NMR signal in the alkyl-C region of the gut material spectrum. Cazemier et al. (1997) report concentrations of short-chain fatty acids including acetate in the guts of a range of plant material-feeding arthropods, including the termite *Mastotermes darwiniensis*, to be mostly less than 50 mM. Such concentrations are probably

Table 2. Carbon and nitrogen concentrations of the different materials. Each value is the mean of three replicates with the standard errors in brackets

Organism	Material	C concentration (mg g^{-1})	N concentration (mg g^{-1})
M. parvus	Food (wood)	450 (3.2)	5 (0.8)
	Guts plus contents	310 (1.6)	42 (1.2)
	Nest carton (aged faeces)	228 (22.4)	4 (0.5)
T. macrothorax	Crop plus contents	98 (1.2)	11 (0.2)
	Hindgut plus contents	78 (0.9)	13 (0.1)
	Mound	46 (0.5)	2 (0.6)
B. marci	Food (leaf litter)	383 (3.6)	16 (0.5)
	Residual food	332 (4.5)	15 (0.1)
	Faeces I[1]	304 (4.3)	13 (0.4)
	Faeces II[1]	203 (0.5)	12 (0.2)

[1] Faeces I and faeces II are the faeces collected after the first and second passages through the gut, respectively.

well below the threshold of detection by solid-state ^{13}C NMR.

There was a very large signal in the carbonyl-C region (160–200 ppm) for the gut material. C from several sources including amide-C in polypeptides and carboxyl-C in organic acids, such as acetic (ethanoic) acid, may be represented in the carbonyl-C region (Table 1). In the absence of strong evidence for acetate from the methyl-C part of the spectrum, it is unlikely that acetate contributed substantially to the carbonyl-C of the gut material. For the gut material, which had a relatively large N concentration (Table 2), the large carbonyl-C can probably be attributed to amide-C in polypeptides from the gut wall. This attribution is consistent with the assignment of the enhanced alkyl-C signal for the gut material to lipid material in the gut wall. On balance, therefore, the enhanced alkyl-C and carbonyl-C signals are attributed to the gut wall, rather than metabolites in the gut.

All materials gave strong signals in the O-alkyl-C (60–90 ppm) and the acetal- and ketal-C (90–110 ppm) regions attributed to polysaccharides, such as cellulose. The relative proportions of the total spectrum represented by the polysaccharide signals declined from the wood to the gut material and again to the nest carton, indicating preferential loss of this component during passage through the gut. It is important to note, however, that substantial polysaccharide signals remained even in the nest carton and, therefore, that utilization of this material by *M. parvus* was far from complete.

The intensity of the distinct signal at 55 ppm increased substantially from the wood to the gut material and again to the carton material. Signals in this region are difficult to assign because both N-alkyl-C in amino acids and methoxyl-C in lignin side-groups appear here. First, in view of the small total N content of the mound material for which the 55 ppm signal was largest, and second, if it is accepted that the larger N concentration of the gut sample is accounted for by polypeptides (giving carbonyl-C signals) rather than free amino acids, the large NMR signal at 55 ppm cannot be assigned to N-alkyl-C and was probably, therefore, methoxyl-C. This assignment is consistent with the relative increase in the intensity of the NMR signal at about 145 ppm, attributed to O-aromatic-C. Both O-aromatic-C and methoxyl-C would be present in lignin and both signals increased in intensity to similar extents with passage through the gut. The methoxyl-C and O-aromatic-C signals are consistent with selective preservation of the lignin component of the wood during passage through the gut, although it is not known whether this is absolute or relative lignin accumulation. The fact that the methoxyl substituent appears to accumulating relative to the phenolic moeities, suggests modification of the lignin associated with passage through the termite gut (Butler & Buckerfield 1979; Cookson 1988; Breznak & Brune 1994; Brune *et al.* 1995). This modification of lignin may allow greater exploitation of the polysaccharide component of the wood.

T. macrothorax (soil-feeding termite)

The samples of the material from *T. macrothorax* had relatively small C concentrations (Table 2), which

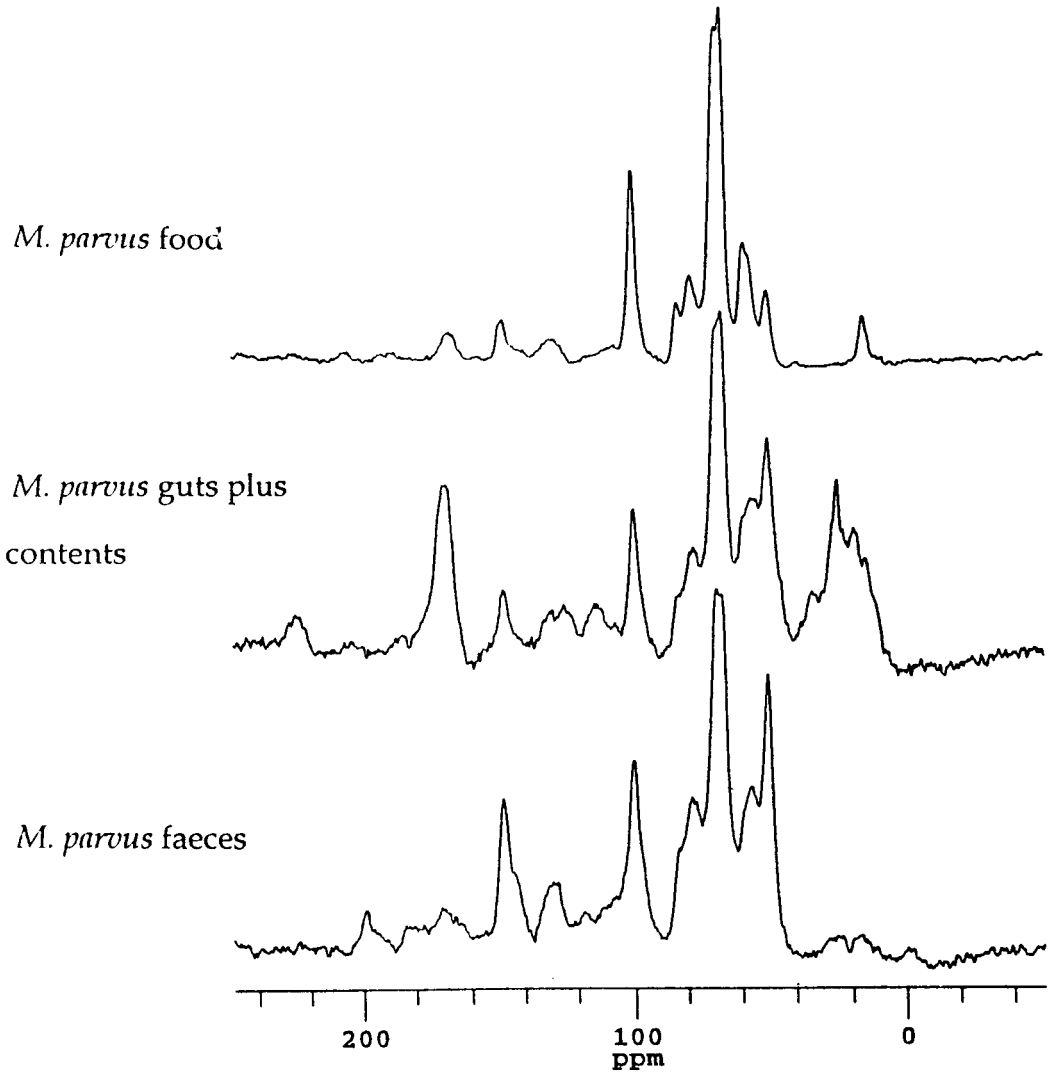

Figure 1. CP MAS ^{13}C NMR spectra for samples of the food (wood), gut plus contents and faeces from the wood-feeding termite *Microcerotermes parvus*.

strongly influenced the quality of the spectra (Figure 2), even though the Fe concentration had been diminished.

The large carbonyl-C (160–200 ppm) signals in the crop and hindgut samples were probably due to the termites' gut tissue for similar reasons to those outlined for *M. parvus.* The mound material, which had a spectrum similar to whole soil samples containing humified organic matter (e.g., Skjemstad et al. 1997), also contained detectable carbonyl-C, so unless the carbonyl-C in the mound material arose solely from organic transformations after it had been incorporated into the mound, it is not possible to assign the carbonyl-C signal solely to the gut tissue rather than the gut contents.

Many studies have shown that the relative intensity of signals in the alkyl- and methyl-C region increase relative to those of carbohydrates as soil organic matter decomposes and becomes further humified (e.g., Hopkins et al. 1993; 1997). This occurs because of selective preservation of some plant components such as waxes and the production of microbial metabolites and components, such as lipids, in the microbial biomass, whilst the carbohydrate component is degraded. Since *T. macrothorax* feeds on soil containing more or less humified organic matter, the alkyl- and methyl-C

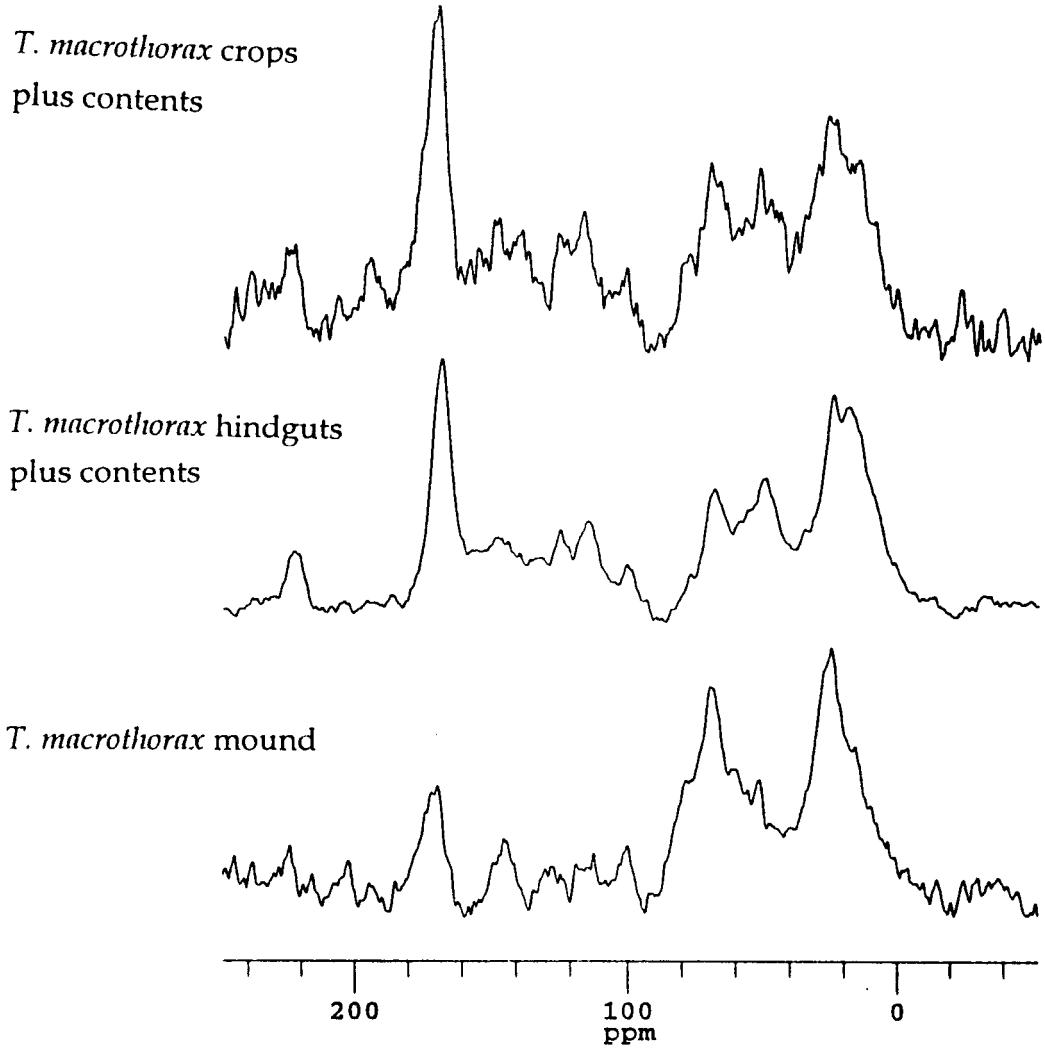

Figure 2. CP MAS ^{13}C NMR spectra for samples of the crops and hindguts plus contents and the mound (aged faeces) from the soil-feeding termite *Thoracotermes macrothorax*.

in both the termites' gut tissue and the soil may have contributed to the signals between 10 and 45 ppm in the crop and hindgut samples. However, the mound material (aged faeces), which would have contained little if any of the gut tissue, also had a strong signal in the methyl- and alkyl-C region. Without spectra for the uningested soil (the precise fraction or fractions consumed, if any, are not known), it is not possible to determine whether passage through the gut or transformations following incorporation in to the mound, or both, led to a change in the concentration of alkyl- and methyl-C.

Signals in the O-alkyl-C region (60–90 ppm) were present in all materials, but were most intense in the mound material. There was a relative decline in the O-alkyl-C between the crop and the hindgut, possibly indicating relative loss of polysaccharide with passage through the gut. However, since it is not possible from the present spectra to separate the contribution made to the alkyl- and methyl-C signal in the gut contents from that made by the gut tissues, it is not possible conclusively to determine whether the O-alkyl-C concentration of the gut plus contents declined relative to that of alkyl- and methyl-C in the gut lumen. It is suggested that passage through the gut was accompanied by a relative decline in polysaccharide. This is consistent with evidence from gut content analysis that cellulosic materials are available to soil-feeders

(Sleaford et al. 1996) and from respirometry that carbohydrates support metabolism (Nunes et al. 1997).

There was a clear signal at about 55 ppm in the hindgut and crop samples, but not in the mound material. As with the *M. parvus*, this may have been due either to methoxyl-C or N-alkyl-C. The fact that there was not a clear O-aromatic-C signal in either the crop or hindgut samples suggests, by contrast with *M. parvus*, that this signal was due to N-alkyl-C in the gut, possibly arising as intermediates in the digestion of polypeptides or microbial metabolites, or both. It is not possible to use the N concentrations (Table 2) or the C : N ratios of the materials (approximately 9 and 6 for the crop and hindgut, respectively, compared to approximately 20 for the mound) to support the suggestion that the crop and hindgut materials had a relatively large amino acid concentration, because there is no estimate of the N contributed from the gut tissues themselves. Nevertheless, the C : N ratios do not discount this possibility.

B. marci (litter-feeding fly larvae)

All the samples from *B. marci* had a sufficiently large C concentration (Table 2) to allow good quality spectra to be recorded (Figure 3). The C concentration did, however, decline slightly from the food to the residual food and to a much greater extent between the food and the faeces after both the first and second passages through the gut (Table 2). These values can be interpreted in terms of C loss during digestion in the gut or microbially-mediated biodegradation of the faeces because the samples were essentially free of C from the gut tissues.

The spectra for the food and the residual food from *B. marci* (Figure 3) were similar apart from slight relative enhancement in the aromatic region (110–160 ppm) following feeding. This observation is consistent with the larvae feeding selectively on the inter-vein regions of leaf material which contain less lignin (Szabo 1974). Such behaviour is typical of many litter-feeding detritivores (Bignell 1989).

After the first passage through the gut the aromatic-C signals were relatively more intense for the faeces than for either the food or the residual food, indicating selective preservation of the lignin-rich fraction. The signal centred at approximately 15 ppm in the food and residual food had declined to a small shoulder in the faeces after the first passage through the gut, whilst the overall signal at about 40 ppm increased in intensity between the food and the faeces after the first passage. This change may be associated with increased microbial colonization of the material in the gut and fresh excrements.

After the second passage through the gut the relative intensities of signals in the aromatic-C region increased markedly whilst those attributed to polysaccharides had declined. This indicates preferential digestion of polysaccharide components and further selective preservation of the lignin components. In view of the fact that the intensities of the aromatic-C signals increased and the polysaccharide-C signals decreased more markedly after the second compared to the first passage through the gut, it is possible that principally mechanical and physical changes occurred initially and that chemical digestion was prevalent during the second passage. This may be because of greater microbial colonisation of the food in the gut during second passage in comparison with first passage, as has been observed in other litter-dwelling animals (Gunnarson & Tunlid 1986), including *B. marci* larvae (J Frouz, H Šantrucková & D Elhottová, pers. com. unpubl.).

Conclusions

We have successfully applied solid-state ^{13}C NMR to investigate the transformations of organic materials during biodegradation associated with three contrasting insects involved in the biodegradation of organic residues. However, the interpretation of the spectra was limited in some cases, first, by inability to distinguish between C in gut tissue and C in gut contents, and second, by the small sizes, or the small C concentrations, or the large concentrations of paramagnetic species of the samples. The problems of small C and large paramagnetic concentrations were particularly associated with the soil-feeding termite, but these difficulties are common to the application of NMR to many types of environmental investigation.

The ecological contrasts between the insects were supported by the NMR evidence, which showed that there was preferential loss of polysaccharide and accumulation of lignin with some modification in the wood-feeding termite; some evidence of loss of polysaccharide in the soil-feeding termite; and preferential feeding on the polysaccharide-rich component of the litter, followed by overall loss of polysaccharide and accumulation of both lignin-derived material occurring to different extents between the first and

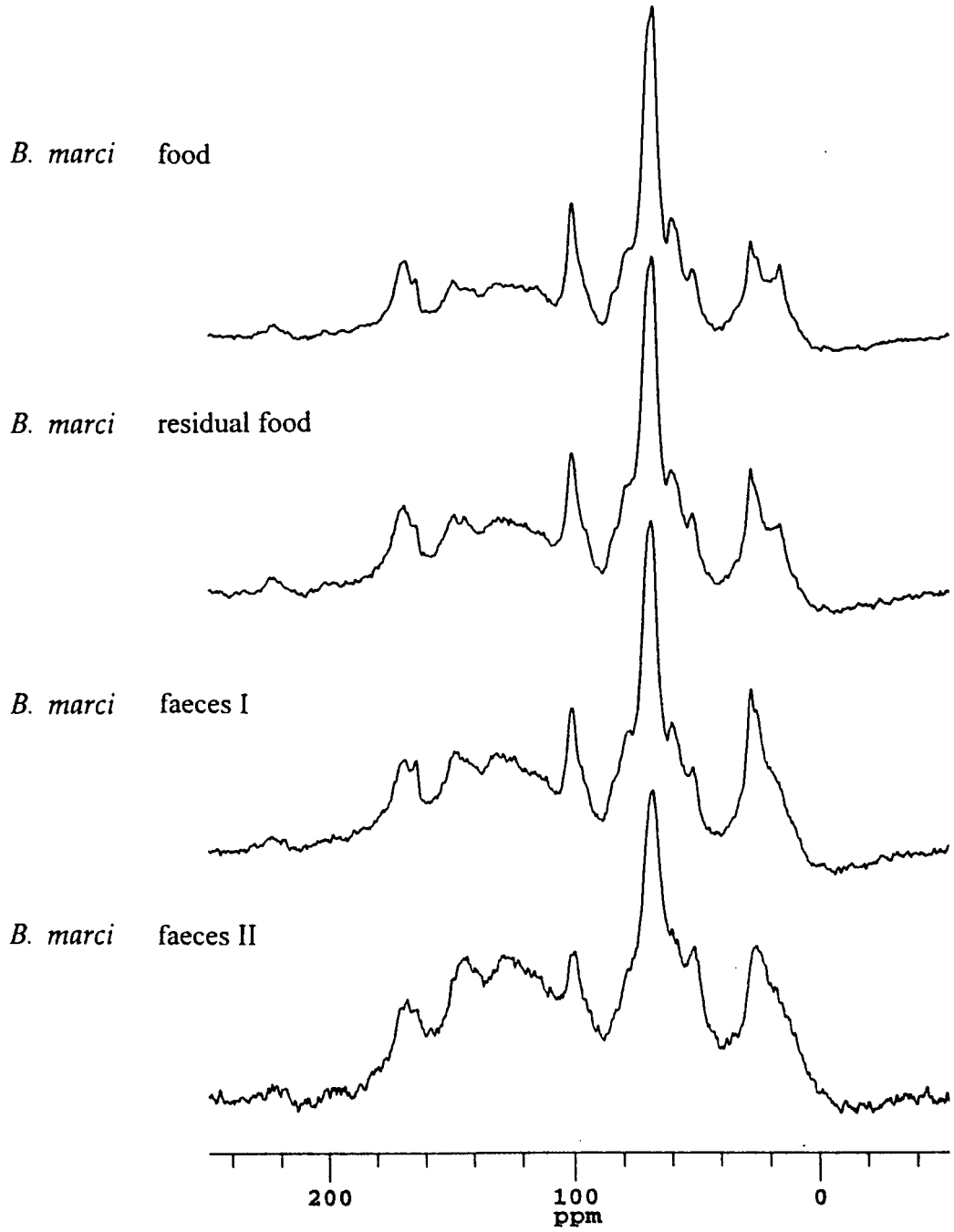

Figure 3. CP MAS ^{13}C NMR spectra for samples of the food (hornbeam leaves), the residual leaves after feeding, the faeces after the first passage through the gut (faeces I) and the faeces after the second passage through the gut (faeces II) from the coprophagous larvae of the dipteran, *Bibio marci*.

second passages through the gut for the coprophagic insect.

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