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Action of the saproxylic scarab larva *Cetonia aurataeformis* (Coleoptera: Scarabaeoidea: Cetoniidae) on woody substrates

E. Micó[a]*, M. Juárez[b], A. Sánchez[b] and E. Galante[a]

[a]*Centro Iberoamericano de la Biodiversidad CIBIO, Universidad de Alicante, Ctra. de San Vicente del Raspeig s/n, 03690 Alicante, España;* [b]*Departamento de Agroquímica y Bioquímica, Universidad de Alicante, Ctra. de San Vicente del Raspeig s/n, 03690 Alicante, España*

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The saproxylic beetle *Cetonia aurataeformis* Curti (Scarabaeoidea: Cetoniidae) is a common Iberian species, whose larvae develop in tree cavities feeding on wood and litter. The aim of this paper is to analyse how the larvae of this cetonid modify the woody substrate by feeding and what the ecological implications on their microhabitat could be. Thermal analysis and infrared spectroscopy have been used to study the changes suffered by different substrates, litter and wood of *Betula alba* and *Quercus pyrenaica*, after digestion by the larvae. Results show that larvae of *C. aurataeformis* are able to digest polysaccharides and lignin producing a residue richer in nutrients than the original substrate and with an organic structure that contains a fraction of lignin that is easier to decompose. The main conclusion is that the action of cetonid larvae on woody substrates could facilitate their use by other saproxylic organisms in natural ecosystems.

Keywords: FTIR; lignin and polysaccharides degradation; nutritional ecology; TG; wood transformation

Introduction

Saproxylic invertebrates comprise the largest component of biodiversity in terrestrial ecosystems (Speight 1989; Schlaghamersky 2003). They are responsible for the mechanical breakdown of woody material (Cavalli and Mason 2003) both directly, by tunnelling and feeding in living trees that are decaying, snags (standing dead trees) and logs (fallen trees, portions of trunk and large branches), and indirectly, through symbiotic relationships with fungi and other microorganisms that humify wood (Speight 1989).

Wood consists of an orderly arrangement of cells with walls composed of varying amounts of cellulose, hemicellulose and lignin. The great diversity of woody plants is reflected in their varied morphology and chemical composition. According to Martin et al. (1991) cellulose digestion has been shown in 78 species of insects from 20 families representing eight orders, including representatives of the Coleoptera Anobiidae, Buprestidae, Cerambycidae and Scarabaeidae families. Different mechanisms have been proposed to account for cellulose digestion in insects, with the exploitation of the cellulolytic capacity of bacteria residing in the insect's hindgut, and the reliance upon fungal cellulases being the most common in Coleoptera (Bayon 1981; Kukor and

*Corresponding author. Email: e.mico@ua.es

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Martin 1986; Kukor et al. 1988; Martin et al. 1991). Lignin also plays a central role in carbon cycling on earth. Despite the fact that a significant fraction of lignocellulose passes through arthropod guts, the fate of lignin in these systems is not well known. Lignin degradation by two insect species, the Asian longhorned beetle (*Anoplophora glabripennis* Motschulsky) and the Pacific dampwood termite (*Zootermopsis angusticollis* (Hagen)), has recently been shown by Geib et al. (2008). However, is commonly accepted that the dietary factors that usually limit growth and reproduction in insects are mainly nitrogen and water (Slansky and Scriber 1985; Nardi et al. 2002). The survival and growth of many arthropods on diets with extremely high carbon to nitrogen (C/N) ratios suggest that these arthropods do not obtain sufficient nitrogen from their diets but must be obtaining additional nitrogen from some other source(s) (Nardi et al. 2002). Little is known about the occurrence of nitrogen fixation in arthropod taxa, but nitrogen fixation in insects with poor diets, like wood, has been proved in termites (Breznak et al. 1973), the Scolytid Coleoptera *Dendroctonus terebrans* (Olivier) (Bridges 1981) and *D. valens* LeConte (Morales-Jimenez et al. 2009), the saproxylic cockroach *Cryptocerus punctulatus* Scudder (Breznak et al. 1974), the scarab *Cetonia aurata* L. (Citernesi et al. 1977) and the stag beetle *Dorcus rectus* (Motschulsky) (Kuranouchi et al. 2006). Suh et al. (2003) showed a widespread association of passalid beetles with endosymbiotic organisms that allow them to ferment and assimilate xylose, and Bayon and Mathelin (1980) described cellulolysis in *Oryctes* scarab larvae.

Recently, Nardi et al. (2002) analysed the significance and role of arthropods in nitrogen fixation in terrestrial ecosystems, and concluded that nitrogen fixation in arthropod guts may represent a significant contribution both to the growth of arthropods and to their ecosystem functions of processing carbon and nitrogen. However, from the functional ecology point of view, little attention has been paid to the role of the saproxylic species in their peculiar microhabitat. Therefore, little is known about how insects living in tree cavities modify the substrate while feeding, and if that may consequently facilitate the fitness of other saproxylic organisms. Jönsson et al. (2004) estimated the amount of nitrogen (N) and phosphorus (P) in wood mould of trunk cavities, and compared the coarse fraction which constitutes frass of the scarab beetle *Osmoderma eremita* (Scopoli) (Coleoptera: Scarabaeoidea: Cetoniidae) larvae with the finer fraction, and found that nutrient richness was higher in frass. The authors concluded that the presence of *O. eremita* larvae in trunk cavities probably increases the nutrient richness, with this being one of the possible explanations why the species richness of saproxylic beetles is higher in hollow oaks where *O. eremita* is present.

The Cetoniidae (commonly known as flower beetles) is a large, cosmopolitan group consisting of about 510 genera and 3600 species. The adults appear to be nectar and pollen feeders and are attracted to plant exudates. The larvae are known to live in decaying vegetable matter and rotten wood (Cassis and Weir 1992; Micó and Galante 2003). There are differences in the substrates exploited by the immature stages of the different cetonid species, however many of them are saproxylic and develop in the hollows of branches and trunks of large trees. In that peculiar microhabitat, larvae exploit rotten wood and organic matter that accumulates in the tree cavities producing high amounts of faeces (Micó and Galante 2003). *Cetonia aurataeformis* Curti (Coleoptera: Scarabaeoidea: Cetoniidae) (Figure 1) is a common species in the Mediterranean region of the Iberian peninsula (see Micó and Galante 2002). As with other cetonid larvae, they develop mainly in rotten logs and trunk cavities, feeding on litter and wood, and produce a high quantity of frass (Micó and Galante 2003), which

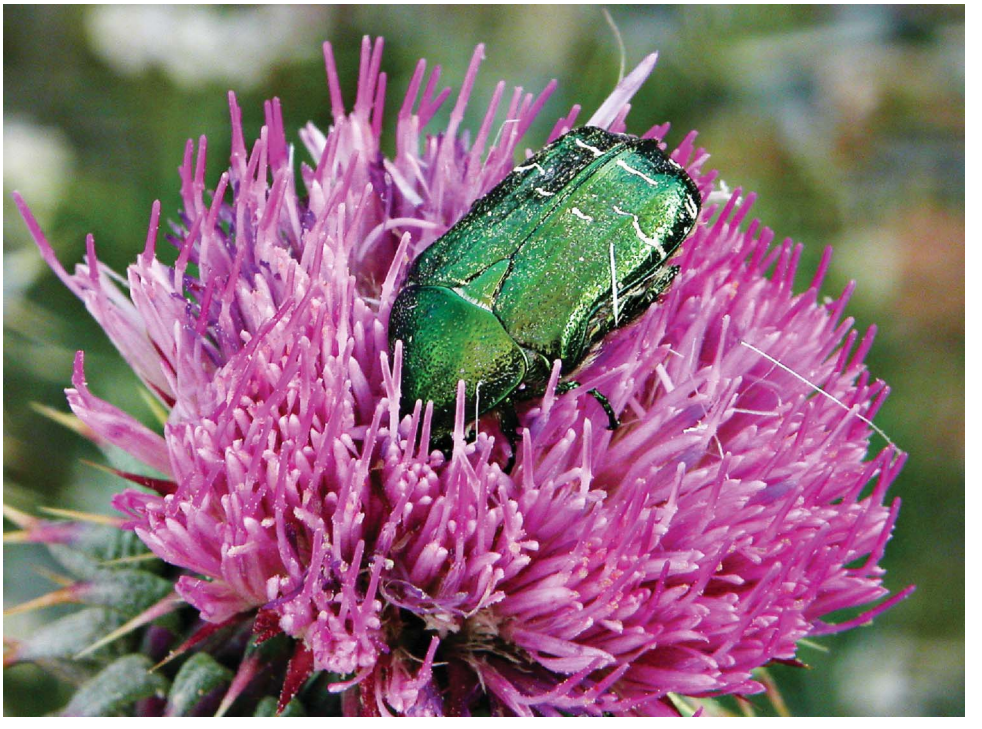

Figure 1. *Cetonia aurataeformis* (Photo: J. Ordoñez, with permission).

remains with the substrate for a long time, at least for more than one year (Micó and Galante, personal observation). This may be one of the first steps in the transformation of some kinds of woody substrates and plant residues, producing organic matter with a composition, consistency and structure that is different to the original substrate. The main question is how the larvae modify the woody substrate and what the implications of their action on their microhabitat might be.

Many studies have used different chemical techniques to characterize and analyse the changes in woods produced by various chemical treatments or attacks by organisms (fungi and bacteria). Thermogravimetry is a well-established technique for studying primary and secondary thermal decomposition of solids and macromolecules from many systems, including woody materials. By measuring weight loss and recording peak decomposition temperatures from thermogravimetric (TG) and derivative thermogravimetric (DTG) (rate of weight loss) profiles, it is possible to extract important compositional information. High temperatures of decomposition can be associated with more resistant organic fractions and more ordered structures when similar organic samples are compared (Lyons et al. 2008). In this way, Genestar and Pons (2008) and Genestar and Cifre (2002) have used thermal analysis to establish the changes in wood of pieces of art from the fifteenth to eighteenth centuries. Infrared spectroscopy is a routinely used technique because it is extremely fast as well as being a non-destructive and non-invasive method for analysing woods (Pandey 1999); it allows the cellulose content in wood and other substrates to be determined (Fackler et al. 2007; Popescu et al. 2007) and establishes the chemical changes produced in wood

during decomposition by fungi (Pandey and Nagveni 2007), bacteria (Gelbrich et al. 2008) and chemical treatments (Mononen et al. 2005).

In this paper, we have used thermal analysis and infrared spectroscopy to study the changes suffered by litter of Mediterranean brushwood and wood of *Betula alba* L. and *Quercus pyrenaica* Willd after its digestion by *Cetonia aurataeformis* larvae in order to ascertain the possible implications of their action on their microhabitat.

Materials and methods

Field observations

In order to estimate the relative abundance of *C. aurataeformis* in trunk cavities in natural ecosystems, we studied a total of 87 trunk cavities belonging to the tree species *Quercus ilex*, *Q. pyrenaica*, *Q. faginea* and *Fraxinus angustifolia* at the National Park of Cabañeros (Ciudad Real, Spain). The survey of the cavities was carried out using "emergence" traps, as in Micó et al. (2010), from February 2009 to February 2010, and was conducted by Micó, Quinto and Galante.

Insect rearing and experimental design

Larvae used for the experiment were reared from eggs from different mating pairs. After egg hatch, a total of 8 to 10 larvae were moved to each of three rearing jars, each containing a different woody substrate: wood of *Betula alba* (BW) from a decaying branch (about 8 cm diameter), wood of *Quercus pyrenaica* (QW) from a decaying branch (about 10 cm diameter) and litter (L) containing leaves and fine woody structures from Mediterranean brushwood, mainly composed of Cistaceae, Labiatae and Fabaceae. Larvae ate and produced frass, which was separated mechanically from the rest of the substrate. We analysed the three substrates used to feed the larvae (BW, QW, L) and the frass produced after the digestion of the three substrates by the *C. aurataeformis* larvae (BF, QF, LF). Samples of the substrate and of the frass produced by the larvae after feeding with the substrate were taken three times during larvae development, so finally a total of nine samples of substrate and nine samples of frass were obtained for analysis.

Sample analysis

Samples were ground and dried at 60°C. Elemental composition was analysed in a Carlo Erba CHNS-O EA1108 apparatus; oxygen (O) content was calculated by difference with the rest of elements and ash (residue, see Table 1) content. Residue was obtained from thermogravimetric data. Copper (Cu), Zinc (Zn), Manganese (Mn), Iron (Fe), Calcium (Ca), Magnesium (Mg), Potassium (K) and Sodium (Na) content were analysed in a Perkin Elmer Optima 4300DV spectrometer using ICP (inductively coupled argon plasma emission spectroscopy); phosphorus (P) was determined using colorimetry with phospho-molybdovanadate at 460 nm (Kitson and Mellon 1944).

In order to measure the weight loss (TG) and the rate of weight loss (DTG) of the samples, we carried out thermal analyses on a Mettler Toledo TGA/SDTA851e/SF/1100 apparatus. A linear heating rate of 10°C min^{-1} was applied for all thermal tests, within the temperature range 25–600°C. The sample

size was about 5 mg and Figure 2 shows DTG versus temperature. The infrared spectra, used to evaluate the chemical changes of the samples, were recorded using a Bruker IFS 66 FTIR spectrophotometer by means of direct measurement with an ATR (attenuated total reflectance)-unit, between 4000 and 600 cm^{-1}. The FTIR (Fourier Transform Infrared Spectroscopy) spectra were baseline-corrected and normalized with the band at about 1028 cm^{-1}, which was set to 1. The band heights were measured from a baseline drawn from 1860 to 780 cm^{-1} (Fackler et al. 2007).

Results

In natural ecosystems, larvae of *Cetonia aurataeformis* are quite common in trunk cavities. We found the presence of this cetonid at 39 of the 87 cavities prospected (see "Materials and methods").

The elemental analysis of samples (Table 1) shows that carbon and oxygen contents differ among the three woody materials, ordered as follows: *Betula alba* wood (BW) > *Quercus pyrenaica* wood (QW) > litter (L). However, the N content for L was about twice that of BW and QW (Table 1).

Comparing the elemental composition of frass with the original substrate used to feed the larvae (Tables 1 and 2), an increase in N content is observed, while C, hydrogen (H) and O content are not modified. Therefore, the C/N ratio decreases in frass, while the O/C and H/C ratios do not vary (Table 1), indicating an enrichment of N in the frass.

The ash content (residue; Table 1) shows that frass has a mineral content higher than that of the original substrate. Moreover, BW has the lowest mineral fraction. This could be related to the higher content of C and the lower mineral content shown by BW in contrast to the other two substrates (Table 1), with the exception of Cu and Zn. The high iron content of QW and L stands out. The mineral content of

Table 1. Composition of substrates (BW: *Betula alba* wood; QW: *Quercus pyrenaica* wood and L: litter) and frass of larvae fed on *Betula alba* wood (BF), *Quercus pyrenaica* wood (QF) and litter (LF).

	BW	QW	L	BF	QF	LF
C (%)	48.8 ± 0.9	44.6 ± 0.6	41.7 ± 0.3	48 ± 2	41 ± 8	40.8 ± 0.3
H (%)	5.9 ± 0.1	5.54 ± 0.09	5.4 ± 0.4	5.6 ± 0.2	5 ± 1	4.9 ± 0.1
N (%)	0.5 ± 0.2	0.47 ± 0.05	1.0 ± 0.1	0.83 ± 0.05	0.8 ± 0.5	1.4 ± 0.1
O (%)	45 ± 1	42 ± 2	39 ± 1	44 ± 3	39 ± 6	32 ± 2
C/N	114 ± 36	95 ± 9	41 ± 4	58 ± 3	64 ± 4	29 ± 3
O/C	0.91 ± 0.04	0.93 ± 0.03	0.93 ± 0.03	0.9 ± 0.1	0.95 ± 0.06	0.78 ± 0.04
H/C	0.122 ± 0.004	0.1242 ± 0.0004	0.13 ± 0.01	0.118 ± 0.003	0.12 ± 0.01	0.120 ± 0.002
Residue (%)	0.11 ± 0.01	8 ± 2	13 ± 2	1.9 ± 0.8	13 ± 2	21 ± 2
P (%)	0.024 ± 0.005	0.03 ± 0.01	0.05 ± 0.01	0.042 ± 0.006	0.07 ± 0.01	0.06 ± 0.01
Ca (%)	0.42 ± 0.03	2.4 ± 0.7	3.5 ± 0.4	0.9 ± 0.1	1.3 ± 0.5	3.9 ± 0.7
Mg (%)	0.04 ± 0.01	0.07 ± 0.02	0.13 ± 0.03	0.077 ± 0.006	0.15 ± 0.06	0.27 ± 0.04
K (%)	0.044 ± 0.005	0.05 ± 0.01	0.08 ± 0.02	0.11 ± 0.01	0.3 ± 0.1	0.17 ± 0.02
Na (%)	0.052 ± 0.009	0.06 ± 0.02	0.056 ± 0.001	0.08 ± 0.02	0.11 ± 0.03	0.06 ± 0.01
Fe (ppm)	0.005 ± 0.002	303 ± 12	1465 ± 299	0.014 ± 0.005	318 ± 60	3423 ± 350
Mn (ppm)	14 ± 4	203 ± 40	72 ± 10	39 ± 9	261 ± 11	112 ± 3
Cu (ppm)	29 ± 4	12 ± 3	17 ± 4	8.4 ± 0.4	16 ± 5	19 ± 5
Zn (ppm)	65 ± 5	9 ± 4	21 ± 3	127 ± 30	25 ± 10	52 ± 15

Table 2. Ratio of element content in frass/element content in susbtrate. BW: *Betula alba* wood; QW: *Quercus pyrenaica* wood; L: litter; BF: frass of larvae fed on *Betula alba*; QF: frass of larvae fed on *Quercus pyrenaica*; LF: frass of larvae fed on litter.

Element	BF/BW	QF/QW	LF/L
C	0.98 ± 0.06	0.9 ± 0.2	0.98 ± 0.01
N	1.9 ± 0.8	1.8 ± 0.1	1.29 ± 0.08
H	0.95 ± 0.03	0.9 ± 0.2	0.95 ± 0.03
O	0.98 ± 0.08	0.9 ± 0.1	0.83 ± 0.05
P	1.8 ± 0.3	1.3 ± 0.2	1.3 ± 0.1
Cu	3 ± 1	1.3 ± 0.1	1.2 ± 0.2
Mn	3 ± 1	1.3 ± 0.3	1.5 ± 0.2
Zn	3 ± 1	3 ± 1	3 ± 1
Fe	3 ± 1	1.1 ± 0.4	2.3 ± 0.9
Ca	3 ± 1	0.6 ± 0.4	1.10 ± 0.07
Mg	2 ± 1	2 ± 1	2.0 ± 0.8
K	2.4 ± 0.6	6 ± 2	2.0 ± 0.3
Na	1.7 ± 0.6	2 ± 1	1.1 ± 0.2

the different substrates studied (Table 1) and the ratio between the content of each element in frass and the original substrate (Table 2) indicate that digestion of the three woody substrates by *C. aurataeformis* larvae produces frass with high content of minerals, N and P.

Thermal analysis of unchanged woods and of woods with different levels of decomposition caused by different factors allows us to establish different temperature ranges at which decomposition occurs (Liodakis et al. 2002; Genestar and Pons 2008) (Figure 2). The first, in the range of 30–100°C, corresponds mainly to the drying process due to the evaporation of moisture. The second, in the range of 120–160°C, is attributed to the evaporation of volatile compounds. The peak, in the range of 200–280°C, corresponds mainly to hemicellulose decomposition, while the next, between 320–370°C, is related to cellulose decomposition. The final peaks, in the range of 370–500°C, correspond to lignin decomposition.

The thermal curves (Figure 2) show that the three woody substrates (BW, QW and L) have a shoulder between 280 and 285°C due to hemicellulose thermal destruction, and a peak between 300 and 325°C which can be attributed to cellulose thermal degradation. *Betula* wood (BW) shows a well-defined peak around 456°C (Figure 2A), which corresponds to the lignin decomposition temperature range (Liodakis et al. 2002; Genestar and Pons 2008), while QW and L show two less-defined peaks (Figures 2B, C). However, the shoulder corresponding to hemicellulose (280–285°C) does not appear in the thermal curves of frass. Furthermore, in the range of lignin decomposition (370–500°C), two peaks occur in frass, one near to 400°C and another at higher temperatures (Figure 2).

Comparing the weight loss of each substrate at the temperature ranges corresponding to the decomposition of polysaccharides (hemicelluloses and cellulose) and lignin (Table 3), BW had a higher weight loss in the polysaccharides region than in the lignin region, while the weight loss in both ranges (polysaccharides and lignin) was similar in QW and L. This could indicate that BW is richer in polysaccharides than

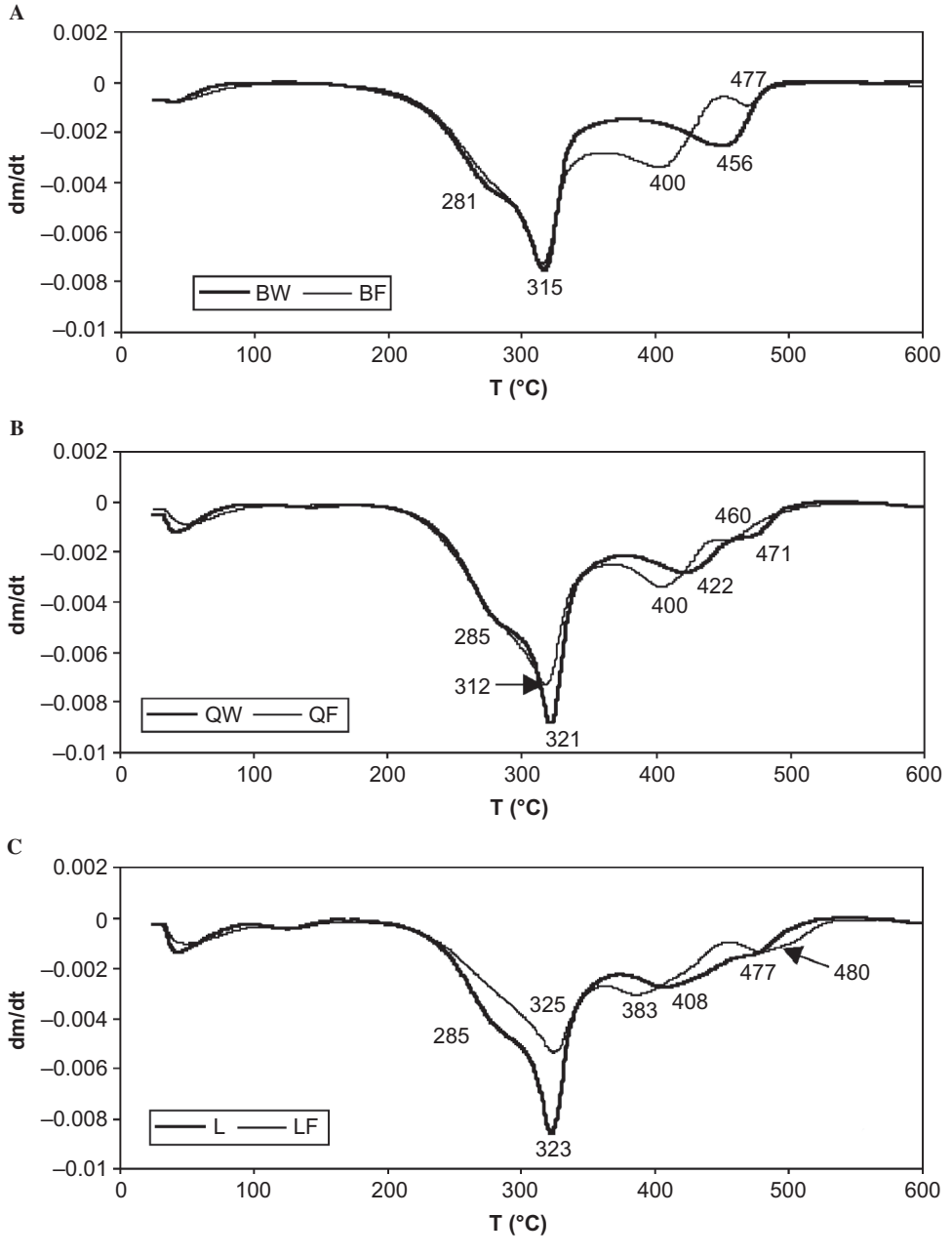

Figure 2. Thermal curves of (A) *Betula alba* wood (BW) and frass (BF), (B) *Quercus pyrenaica* wood (QW) and frass (QF), (C) litter (L) and frass (LF). Y-axis shows the mass change in respect to temperature (dm/dt: derivation of mass in respect to temperature).

the other woody substrates. Frass of larvae fed on *Q. pyrenaica* (QF) and litter (LF) also showed similar weight loss in both regions, polysaccharides and lignin, while BF showed higher weight loss in lignin than in polysaccharides.

FTIR spectra of the three woody substrates (Figure 3) show a large band corresponding to OH. . .O inter- and intramolecular hydrogen bonds (1), and an outstanding band corresponding to symmetrical CH stretching in aromatic methoxyl groups and in methyl and methylene groups of side chains (2). Furthermore, in the "fingerprint region" of wood (1800–600 cm^{-1}) a group of well-defined bands is observed assigned to different lignin groups, such as bands 5 and 6, to lignin and polysaccharides, such as bands 7, 8, 10, 12, 14 and 16, and to polysaccharides only, such as bands 3, 9, 13 and 17 (see also Table 4).

The main differences among the spectra of the substrates are observed in band number 5 (Figure 3, Table 5), related to rings of syringyl (lignin) (Table 4), which are very apparent in the spectrum for L and lower for BW. Band number 10, related to cellulose and syringyl (Table 4), is very high for the L spectrum, while QW and BW show a lower intensity for this band. The spectrum of BW shows a high intensity for band number 3, corresponding to hemicellulose (Table 4), however the spectrum

Table 3. Weight loss (%) at different ranges of temperature. BW: *Betula alba* wood; QW: *Quercus pyrenaica* wood; L: litter; BF: frass of larvae fed on *Betula alba*; QF: frass of larvae fed on *Quercus pyrenaica*; LF: frass of larvae fed on litter.

Sample	Polysaccharides	Lignin	Total weight loss
BW	52 ± 2	40 ± 2	92 ± 3
BF	44 ± 1	49 ± 3	93 ± 5
QW	47 ± 5	42 ± 2	89 ± 3
QF	49 ± 5	44 ± 1	93 ± 3
L	43 ± 1	40 ± 2	83 ± 3
LF	36 ± 2	38 ± 2	74 ± 2

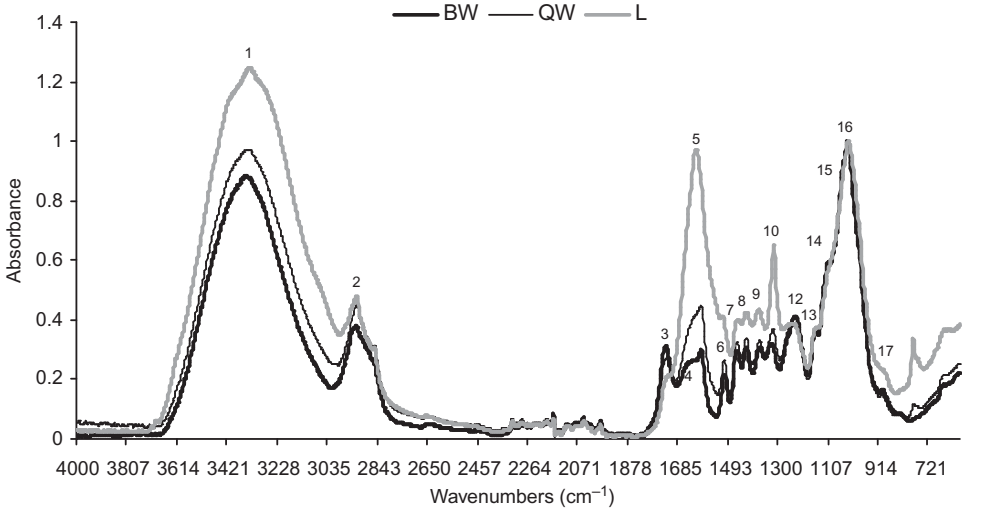

Figure 3. FTIR spectra of litter (L), *Betula alba* wood (BW) and *Quercus pyrenaica* wood (QW), normalized with the band about 1028 cm^{-1}.

Table 4. Assignment of bands in FTIR spectra according to the literature data.

Wavenumber (cm^{-1})	Band assignment	Band
3350–3340	OH. . .O inter- and intramolecular hydrogen bonds[1,2]	1
2925–2920	Symmetrical CH stretching in aromatic methoxyl groups and in methyl and methylene groups of side chains[1,2]	2
	C=O stretch in unconjugated ketones (hemicellulose)[1–9]	
1740–1720	C=C stretching of the aromatic ring of syringyl (lignin)[1–9]	3
1610–1590	C=C stretching of the aromatic ring of syringyl (lignin)[1–9]	5
1515–1505	C–H asymmetric deformation in $–OCH_3$, CH_2 in pyran ring symmetrical scissoring[1–9]	6
1470–1455	C–H asymmetric deformation in $–OCH_3$[1–9]	7
1430–1422	CH bending in cellulose I and cellulose II and hemicellulose[1–9]	8
1375–1365	C–O vibrations in syringyl derivatives, CH in–plane bending in cellulose I and cellulose II[1–9]	9
1335–1320	Guaiacyl ring breathing, C–O linkage in guaiacyl aromatic methoxyl groups[1–9]	10
1267	Syringyl ring breathing and C–O stretching in lignin and xylan[1–9]	11
1235–1230	C–O–C asymmetric stretching in cellulose I and cellulose II[1–9]	12
1162–1125	Aromatic C–H in–plane deformation (typical for syringyl units), C=O stretch[1–9]	13
1120	C–O valence vibration mainly from C–OH[1–9]	14
1060–1015	C–O stretching in cellulose I and cellulose II[1–9]	16
1047	C–H deformation in cellulose[1–9]	15
898		17

Notes: [1]Popescu et al. (2007); [2]Popescu et al. (2010); [3]Harrington et al. (1964); [4]Schultz and Glasser (1986); [5]Collier et al. (1992); [6]Rodrigues et al. (1998); [7]Faix et al. (1998); [8]Pandey and Pitman (2003); [9]Mononen et al. (2005).

of L shows a low intensity for this band (Figure 3, Table 5). The ratios between the intensities of band number 6 of lignin (1504/1508/1513 cm^{-1}) and the bands corresponding to polysaccharides (band number 3: 1734/1731/1728 cm^{-1}, band number 9: 1367/1371/1373 cm^{-1}, band number 13: 1155/1149/1153 cm^{-1} and band number 17: 898/896/897 cm^{-1}) (Table 6) are mostly higher for L, while QW and BW show similar and/or lower values. These values could indicate that litter is the substrate that is less rich in polysaccharides. The ratio between the intensities of band numbers 5 and 6, corresponding to syringyl and guaiacyl respectively (I_5/I_6, Table 6), shows a higher value for L while QW and BW show similar values.

The low values of the ratio between bands associated with lignin (6) and polysaccharides (3 and 13) in the FTIR spectra (Table 6) confirm the results obtained with the thermal analysis (Table 3), indicating that BW is the substrate richest in polysaccharides. Furthermore, it can be concluded that hemicellulose is the main polysaccharide in BW as the intensity of band number 3 of the FTIR spectra is the highest (Figure 3, Table 5); this is also in agreement with the shoulder near to 285°C in thermal analysis.

Table 5. Relative intensities of the main absortions bands in FTIR spectra. BW: *Betula alba* wood; QW: *Quercus pyrenaica* wood; L: litter; BF: frass of larvae fed on *Betula alba*; QF: frass of larvae fed on *Quercus pyrenaica*; LF: frass of larvae fed on litter.

Bands	BW	BF	QW	QF	L	LF
3	11 ± 1	4.6 ± 0.8	9 ± 1	2.4 ± 0.4	4.89 ± 0.03	3.4 ± 0.7
5	10 ± 1	12.8 ± 0.6	15 ± 2	15 ± 2	25 ± 3	19 ± 1
6	7 ± 1	8.7 ± 0.2	8.1 ± 0.7	10 ± 1	9.5 ± 0.4	10.9 ± 0.6
7	9.9 ± 0.1	10.5 ± 0.8	10.0 ± 0.8	10.6 ± 0.1	9.0 ± 0.3	11 ± 1
8	10.3 ± 0.6	11.2 ± 0.3	10.52 ± 0.09	12.2 ± 0.6	9.5 ± 0.3	12 ± 1
9	11 ± 2	8.8 ± 0.2	10.1 ± 0.4	9.4 ± 0.4	9.5 ± 0.4	10.5 ± 0.5
10	11 ± 1	10.2 ± 0.2	11 ± 1	10 ± 1	15.3 ± 0.5	11.7 ± 0.6
11	–	9.3 ± 0.3	–	8.6 ± 0.5	–	7.5 ± 0.4
12	14.0 ± 0.5	10.2 ± 0.5	13 ± 1	8.6 ± 0.6	7.84 ± 0.03	6.9 ± 0.6
13	11.9 ± 0.4	10.0 ± 0.7	10.6 ± 0.3	10.3 ± 0.3	7.0 ± 0.9	7.4 ± 0.8
17	4 ± 0.2	3.5 ± 0.8	2.8 ± 0.7	3.2 ± 0.2	2.3 ± 0.9	–

Note: Data are given as a percentage of the sum of the heights of the main FTIR bands in the region 1800 to 600 cm^{-1}.

Table 6. Ratios between the associated intensities of lignin (bands 5, 6) and of polysaccharides (3, 9, 13, 17) in FTIR spectra. BW: *Betula alba* wood; QW: *Quercus pyrenaica* wood; L: litter; BF: frass of larvae fed on *Betula alba*; QF: frass of larvae fed on *Quercus pyrenaica*; LF: frass of larvae fed on litter.

Ratios	BW	BF	QW	QF	L	LF
I_6/I_3	0.7 ± 0.2	1.9 ± 0.3	0.9 ± 0.1	4 ± 1	1.94 ± 0.08	3.3 ± 0.8
I_6/I_9	0.7 ± 0.2	0.99 ± 0.04	0.8 ± 0.1	1.0 ± 0.1	0.996 ± 0.006	1.04 ± 0.02
I_6/I_{13}	0.6 ± 0.1	0.87 ± 0.08	0.77 ± 0.08	1.0 ± 0.1	1.4 ± 0.1	1.5 ± 0.3
I_6/I_{17}	1.7 ± 0.4	2.6 ± 0.6	3 ± 1	2.8 ± 0.3	4 ± 2	
I_5/I_6	1.5 ± 0.3	1.5 ± 0.1	1.8 ± 0.3	1.55 ± 0.07	2.6 ± 0.4	1.7 ± 0.2

When FTIR spectra of frass are compared with those of the original substrates, a reduction in the intensity of band number 3, associated with hemicellulose (Figure 4, Table 5), is observed after digestion, being almost absent in frass. The intensity of band number 9, related to cellulose and hemicelluose, tends to be lower in frass than in the original substrate. In the region between 1180 and 1280 cm^{-1} (Figure 4, Table 5), while band number 11, corresponding to guaiacyl (Popescu et al. 2007), is not present in the substrate, this band is well defined in frass. However, band number 12, associated with syringyl and cellulose (Popescu et al. 2007), shows a higher intensity in substrates than in frass. The band associated with rings of guaiacyl (band 6; Figure 4) shows a relative intensity higher in frass than in the substrates (Table 5). Band number 5, corresponding to syringyl rings (Popescu et al. 2007), increases its relative intensity in BF in relation to BW, while this band is lower or equal in LF and QF (Table 5). Band number 10 (Figure 4, Table 5) also has lower relative intensity after the digestion of litter in LF.

On the other hand, the ratio between the intensity of band number 6, characteristic of lignin (Popescu et al. 2010), and band number 3, associated with hemicellulose, is higher in frass when compared with the original substrate (Table 6). This indicates that frass has a lower content in hemicellulose than the substrate. However, for the rest of the polysaccharides, the ratio varies depending on the substrate. Meanwhile, the intensities of bands around 1590 and 1504 cm^{-1} (I_5/I_6), characteristic of the relation between the structure of syringyl and guaiacyl (Mononen et al. 2005), are similar for BF and QF when compared with the original substrates (Table 6), being lower for LF.

Discussion

The analysis of both substrate and frass of *C. aurataeformis* larvae shows an enrichment of frass in minerals, N and P (Tables 1 and 2). Considering that the substrates used (BW, QW and L) have a high C/N ratio (Table 1), and that absorbable nitrogen is a limiting factor in insect growth (Hosking and Hutcheson 1979; Haack and Slansky 1985; Dajoz 1998), our results suggest a possible fixation of nitrogen by *C. aurataeformis* larvae as the most suitable explanation of this nitrogen enrichment of frass. This is in agreement with Citernesi et al. (1977), who confirmed the ability of N fixation in other *Cetonia* species' larvae using a ethyne (C_2H_2) reduction assay. This phenomenon has also been proved in other saproxylic insects (Breznak et al. 1973, 1974; Bridges 1981; Kuranouchi et al. 2006; Morales-Jimenez et al. 2009).

Analysing both substrate and frass, our results are thus also in agreement with Jönsson et al. (2004), who found that nutrient richness was higher in the frass of the cetonid *Osmoderma eremita* than in the finer section of their inhabiting holes. However, what is the fate of lignin and polysaccharides of the woody substrates after the action of larvae?

When comparing the weight loss corresponding to thermal decomposition of polysaccharides and lignin of the different substrates (Table 3), similar weight loss is produced by lignin and polysaccharides in QW, L and frass (QF and LF), allowing us to conclude that larvae of *C. aurataeformis* decompose polysaccharides and lignin. However, BW has a higher weight loss in the region corresponding to polysaccharides than that corresponding to lignin. When analysing BF, a higher loss in the region of lignin compared with that of polysaccharides is observed (Table 3), indicating that larvae fed with *Betula* (BW) have decomposed proportionately more polysaccharides

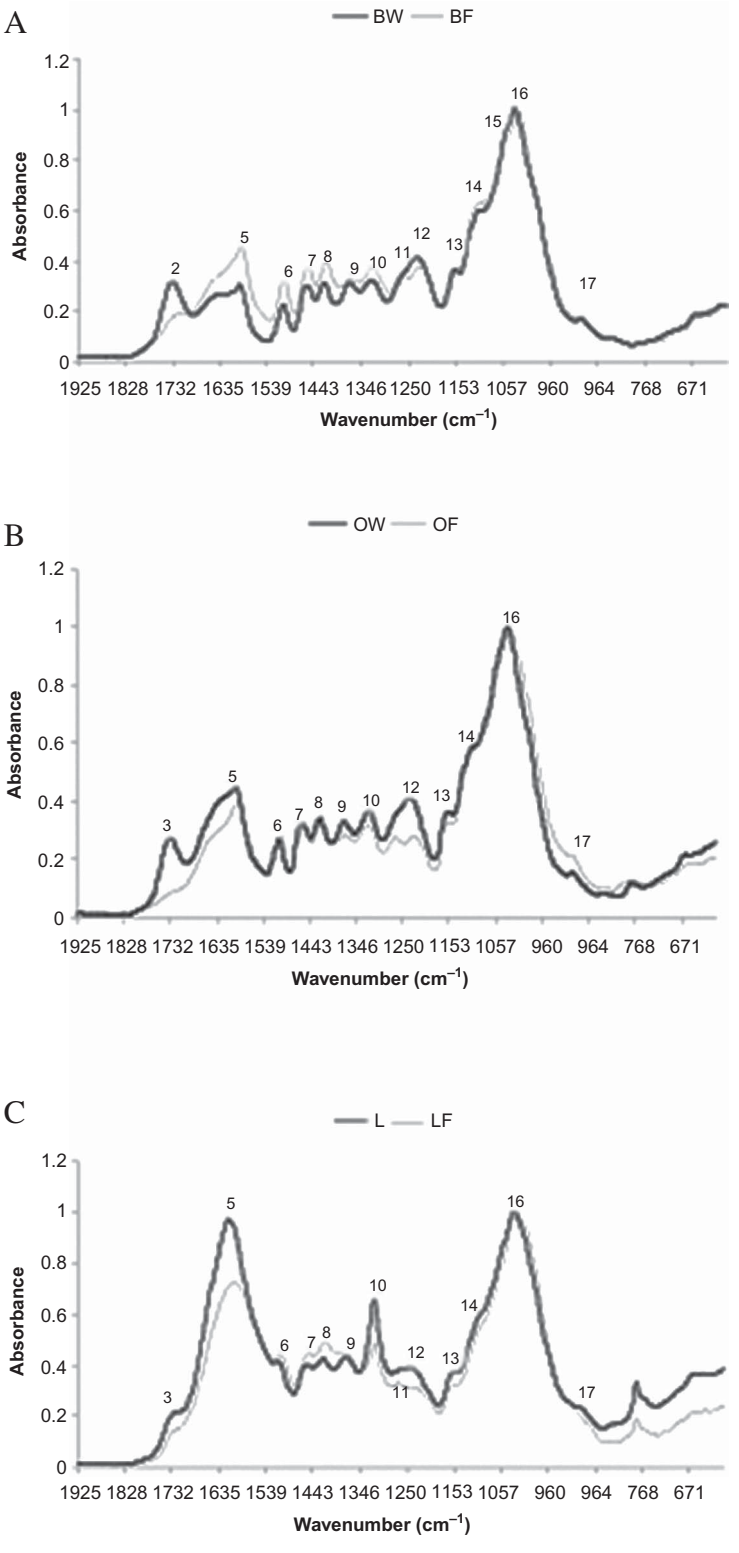

Figure 4. FTIR spectra of frass and substrates, (A) *Betula alba*, (B) *Quercus pyrenaica*, (C) litter, normalized with the band about 1028 cm^{-1}.

than lignin, probably due to the higher content in hemicellulose of BW and the lower structural stability of the hemicellulose than lignin.

The digestion of hemicellulose by larvae of *C. aurataeformis* occurs, and is deduced by the disappearance of the shoulder at 285°C in thermal curves of frass (Figure 2), together with the reduction in intensity of band number 3, associated with hemicellulose in FTIR spectra of frass (Figure 4, Table 5), and with the higher I_6/I_3 ratio obtained for frass compared with those obtained for the substrates (Table 6). Our results are in agreement with Bayon and Mathelin (1980), who demonstrated the existence of cellulolysis in the scarab beetle *Oryctes* (Scarabaeoidea: Dynastinae) as well as the absorption of the products of cellulolysis.

The digestion of polysaccharides by larvae of *C. aurataeformis* can also explain the presence of band number 11 (associated with guaiacyl (Popescu et al. 2007)) in frass, probably caused by the disappearance of the polysaccharides of band number 12 (associated with syringyl and cellulose (Pandey and Pitman 2003; Popescu et al. 2007)), whose relative intensity is conspicuously lower in frass (Table 5) revealing band number 11. The results obtained by FTIR are similar to those of Fackler et al. (2007), Pandey and Pitman (2003) and Gelbrich et al. (2008) about the degradation by different fungi (*Coniophora puteana* (Schumach.) P. Karst., *Coriolus versicolor* Quél, *Phanerochaete chrysosporium* Burdsall, *Ceriporiopsis subvermispora*, *Gloeophyllum traveum* (Pers.) Murrill, *Poria placenta* (Fr.) Cook, *Trametes versicolor* (L.:Fr.) Quél, *Polyporus meliae* (Underw.) Murrill) and bacteria of different woods (*Pinus* spp., *Picea* spp., *Quercus* spp., *Fagus sylvatica* L., *Hevea brasiliensis* (Willd. ex A. Juss) Müll. Arg). These studies have established that the degradation of polysaccharides produces a residue that is richer in lignin and shows the following modifications in FTIR spectra: (1) reduction in the intensities in the bands of FTIR spectra associated with polysaccharides and an increase in the intensity of bands associated with lignin, (2) the splitting of the band around 1244 cm^{-1} (12), and (3) the increase in ratios between the intensity of the band associated with lignin and the bands associated with polysaccharides (I_6/I_3, I_6/I_9, I_6/I_{13}, I_6/I_{17}). On the contrary, studies about degradation of lignin by light in different woods (*Phyllostachys pubescens* (Mazel), *Hevea brasiliensis*, *Betula pendula* Roth, *Pinus roxburghii* Sargent and *Pinus sylvestris* L.), show a decrease in the intensity of bands associated with lignin in the FTIR spectra but splitting of band number 12 is not observed, while the bands associated with polysaccharides have few changes (Pandey and Vuorinen 2008; Wang and Ren 2009).

The change of the distribution of peaks in the range 370–500°C of the thermal curves of frass regarding the substrates (Figure 2) shows an important transformation of lignin and indicates the presence of new structures with lower and higher stabilities than the initial one.

The results of the present study show that larvae of *C. aurataeformis* are able to digest polysaccharides and lignin, producing a residue richer in nutrients than the original substrate and with an organic structure containing a fraction of lignin that is easier to decompose. We conclude that the action of the cetonid larvae on the substrate could consequently facilitate its use by other saproxylic organisms in natural ecosystems, especially, if we consider the high abundance of this species in trunk cavities (45% of cavities prospected in natural ecosystems). Further understanding of these systems will contribute to our knowledge of the ecological roles that these insects play in carbon cycling in natural systems and the importance of species interactions in the

peculiar microhabitats of trunk cavities. However, much work across many disciplines will be required to elucidate further the biocomplexity of the saproxylic community.

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