Original article

Chemical transformation of *Quercus* wood by *Cetonia* larvae (Coleoptera: Cetoniidae): An improvement of carbon and nitrogen available in saproxylic environments

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Abstract

Chemical changes to *Quercus* wood caused by the larvae of the saproxylic beetle *Cetonia aurataeformis* have been evaluated using elemental analysis, thermal analysis, infrared spectroscopy and nuclear magnetic resonance. Faeces of *C. aurataeformis* from *Quercus rotundifolia* tree hollows and faeces of larval *C. aurataeformis* reared under laboratory conditions were collected and analysed. The results suggest that larval *C. aurataeformis* are able to decompose the polysaccharides in *Q. rotundifolia* wood, producing a residue with higher concentration of N and P, and organic structures with different stability than those found in wood. The higher N and P concentrations and the reduction of the particle size would facilitate the activity of decomposing microorganisms on *C. aurataeformis* faeces. The comparison of the faeces from tree hollows with those from the larvae reared in the laboratory revealed that both N and P concentrations and the stability of organic structures in the region of lignin increased with the amount of time that faeces were in *Q. rotundifolia* hollows, while the stability of the organic structures in the polysaccharides region decreased. The lower stability of organic compounds in faeces gives rise to an increase of soluble organic matter with higher N concentration than that solubilised from the wood the beetles feed on. Moreover, faeces collected in *Q. rotundifolia* hollows showed the highest values of humification and aromaticity, determined by the spectroscopic indices of soluble organic matter, highlighting the action of this cetonid in the carbon and nitrogen cycle in saproxylic environments.

Keywords: Wood transformation; Saproxylic; FTIR; TG; Humification index; Tree hollows

1 Introduction

Forests make up the major percentage of terrestrial ecosystems and woody debris is recognised as an important long-term pool of forest carbon [29]. Despite being key to forest productivity, the understanding of how saproxylic organisms affect wood chemistry and organic matter stability during wood decomposition is still limited (see Refs. [47,50].

Among saproxylic insects, beetles make up the biggest part of saproxylic diversity and are primarily responsible for the mechanical breakdown of woody material [4], both directly, by tunnelling and feeding, and indirectly, through symbiotic relationships with fungi and other microorganisms that humify wood [45].

Many studies have shown increases in nutrient concentrations, especially nitrogen (N) and phosphorus (P), as wood decomposes [48]. A higher concentration of P and N than in surrounding soils was found in fresh earthworm casts [22], in freshly constructed termite mound materials [27], and in nests of wood ants [8]. Also, higher concentration of N and P than that of wood has been found in faeces of humivorous cetonid beetle larvae *Pachnoda ephippiata* [24,25], and in the faeces of the wood-feeding scarabaeid beetle larvae *Osmoderma eremita* [18] and *Cetonia aurataeformis* [31]. Moreover, numerous farmers from Africa, South America and Australia have long recognised the benefits of insects such as termites to crop production [48].

The saproxylic beetle *Cetonia aurataeformis* Curti (Coleoptera: Scarabaeoidea: Cetoniidae) is a common Iberian species, the larvae of which develop mainly in tree hollows while feeding on fragments of wood and litter. These larvae produce a high volume of easily distinguishable faeces, which remain with the substrate for more than a year [43]. Micó et al. [31] reared *C. aurataeformis* larvae in three different woody substrates (litter, *Betula alba* wood, and *Quercus pyrenaica* wood) in laboratory conditions and concluded that larvae were able to digest polysaccharides and lignin, producing a residue richer in nutrients than the original substrate with an organic structure that contains a fraction of lignin that is easier to decompose. On the other hand, Micó et al. [33] showed that tree hollows where larval activity of Cetoniidae and *Cerambyx* (Cerambycidae) species was present contained a greater diversity of saproxylic beetles than those where these species were absent, and greater beetle richness and abundance were related to higher amounts of assimilable carbon and phosphorous. Moreover, Sánchez-Galván et al. [43] showed that a substrate enriched with larval cetonid faeces improved the development and fitness of the saproxylic syrphid *Myathropa florae* (L.) (Diptera; Syrphidae).

Although there is evidence of the chemical transformation of woody substrate by these saproxylic larvae, there is still very little information about the extent to which their activity affects C and N cycles, both in laboratory and natural conditions. Moreover, studies about the connection between the actions of saproxylic insects and soil fertility (increase of C and N availability in saproxylic environment and soils) are still scarce, despite their relevance for comprehension of nutrient cycles in forest ecosystems.

There are different methods used to analyse and characterise the changes of different organic materials throughout the carbon cycle. Thermogravimetry is a well-established technique for studying primary and secondary thermal decomposition of solids and macromolecules from many systems, including woody materials [11,31], sludges, composts [28], humic acids [2], and organic matter in solution [38]. Infrared spectroscopy is another routinely-used technique because it is extremely fast, as well as being a non-destructive and non-invasive method for analysing chemical changes by decomposition of organic residues in natural ecosystems and in the composting process [10,31,37]. Solid-state ^{13}C nuclear magnetic resonance spectroscopy with cross-polarisation and magic angle spinning (CPMAS ^{13}C NMR) allows information to be obtained directly and in a non-destructive way from the carbon components of an entire sample without any chemical or physical fractionation, and it is well suited to the characterisation of organic matter, including wood, litter, lignite, humic substances and compost [1,2,16,17,30,47,51].

Taking into account that N is the most limiting nutrient in a saproxylic environment, we aimed to analyse the connection between the actions of saproxylic insects and soil fertility. For this purpose, we used thermal analysis, infrared spectroscopy, solid-state ^{13}C nuclear magnetic resonance, ultraviolet-visible (UV-Vis) spectroscopy and fluorescence spectrometry to study the changes to *Quercus rotundifolia* wood after its digestion by larval *C. aurataeformis* in saproxylic environments, such as in tree hollows of *Q. rotundifolia* in nature and under laboratory conditions. We aimed to determine: i) whether the alteration of *Q. rotundifolia* by larvae of *C. aurataeformis* was influenced by the development conditions of the larvae (laboratory and nature); ii) the humification grade of the organic matter in faeces of *C. aurataeformis*; iii) the solubility/availability of C and N in faeces of *C. aurataeformis*, and the possible effects of this faeces on microbial activity in soil.

With the achievement of these aims we anticipate a better comprehension of the effect of *C. aurataeformis* on nutrient cycles in saproxylic environments.

2 Material and methods

2.1 Insect rearing and experimental design

Larvae of *C. aurataeformis* were fed on dead wood of *Q. rotundifolia* (henceforth QW) from Cabañeros National Park, a protected area of 40,856 ha located in central Spain (39° 23′ 47″ N; 4° 29′ 14″ W) with altitude varying between 560 and 1448 m. The climate is Continental Mediterranean and the annual precipitation averages between 500 and 750 mm. The average annual temperature varies from 12.9 to 15.6 °C and the average monthly temperature fluctuates between 3.9 °C (December) and 23.8 °C (July); extreme temperatures of over 40 °C in summer and below −12 °C in winter are possible. The park is constituted by extensive areas of well-preserved Mediterranean landscape with various woodland types, where *Quercus* species (*Q. rotundifolia*, *Q. suber* and *Q. pyrenaica*) are the main representatives [42] and [32]. Larvae used for the experiment were reared from eggs. Ten larvae were moved to a rearing jar containing wood of *Q. rotundifolia* from a decaying branch about 10 cm diameter as in Micó et al. [31]. Larvae ate the wood and produced faeces (henceforth QFL) that were separated mechanically from the rest of the substrate three times throughout the development of the larvae. At the end of this time, the composition of wood and faeces was determined in order to discover the effect of larvae digestion on the woody substrate.

For comparisons with natural conditions, we collected and analysed the faeces of *C. aurataeformis* from thirteen tree hollows of *Q. rotundifolia* in Cabañeros National Park (henceforth QFH). The main feeding resource for larvae in those tree hollows was both the rotten walls of the cavity and the particulate woody material, including frass, that accumulates at the bottom. This cetonid is one of the most abundant saproxylic species in tree hollows in the Mediterranean region of the Iberian Peninsula; more than 90 larvae of different instars have been found in a single tree hollow (personal observation). Consequently, cetonid faeces are very abundant in tree hollows and easily distinguishable (Fig. 1).

Fig. 1 The amount and shape of faeces of larval *Cetonia* in a tree hollow.

alt-text: Fig. 1

2.2 Analysis of chemical composition of *Quercus rotundifolia* wood and of *Cetonia aurateformis* faeces

Samples were ground and dried at 60 °C. Elemental composition was analysed in a Carlo Erba CHNS-O EA1108 apparatus, and oxygen concentration was calculated by difference with the other elements and ash concentration. Concentration in ashes was obtained from thermogravimetric data. Cu, Zn, Mn, Fe, Ca, Mg, K and Na concentration were analysed in a Perkin Elmer Optima 4300DV spectrometer using inductively coupled argon plasma emission spectroscopy (ICP-OES), and P was determined using colorimetry with phosphomolybdovanadate at 460 nm [20].

Thermal analyses were carried out 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. Sample size was about 5 mg. Infrared spectra were recorded using a BRUKER IFS 66 FTIR spectrophotometer for direct measurement with an ATR-unit, between 4000 and 600 cm^{-1}. The FTIR spectra were baseline corrected and normalised to the highest peak, so that the absorbance of the highest peak was set to 1.0. The band heights were measured from a base line drawn from 1860 to 750 cm^{-1} [7].

Solid-state ^{13}C NMR experiments were performed on a Bruker Avance DRX500 operating at 125.75 MHz. Samples were packed into a 4 mm-diameter cylindrical zirconia rotor with Kel-F end-caps and spun at 10000 ± 100 Hz. A conventional CPMAS pulse sequence [53] was used with a 1.0 ms contact time. Between 2000 and 5000 scans were accumulated with a pulse delay of 1.5 s. Line broadening was adjusted to 50 Hz. Dipolar dephasing (DD) spectra were generated with a decoupling delay of 45 μs between cross-polarisation and data acquisition [41]. Spectral distributions (the distribution of total signal intensity among various chemical shift ranges) were calculated by integrating the signal intensity in seven chemical shift regions: carbonyl (210–165 ppm), O-aromatic (165–145 ppm), aromatic (145–110 ppm), O_2-alkyl (110–95 ppm), O-alkyl (95–60 ppm), N-alkyl/methoxy (60–45 ppm) and alkyl (45–10 ppm) [3]. Although there are limitations to the quantitative reliability of CPMAS spectra, it is appropriate to use NMR to compare intensity distributions and study structural features when samples do not differ widely in composition, as was the case for our study [47].

2.3 Study of humification grade of *Cetonia aurataeformis* faeces and their effect on soil respiration

2.3.1 Extraction and spectral characterisation of soluble organic matter

Soluble organic matter (SOM) was extracted from each sample (QW, QFL, QFH) by adding 35 mL of deionised-distilled water to 1 g of sample in a plastic bottle and shaking for 1 h on an orbital shaker. The bottle was then centrifuged at 2900 *g* for 10 min, and the supernatant was filtered through a 0.45 μm syringe filter. The extraction period was selected to minimise microbial SOM alteration during extraction [54]. An aliquot of 10 mL was lyophilised for the elemental analysis. Dilutions from 1:04 to 1:70 were prepared with the remaining filtrate.

UV-Vis absorption spectra of the extracts from 200 to 500 nm were obtained using a JASCO V-630 spectrophotometer and a 1 cm quartz cuvette. The ratio of absorbances at 253 and 203 nm (E_{ET}/E_{Bz}), corresponding to the electron-transfer band (ET) and the benzenoid band (Bz) of benzene UV light absorption, respectively [21], the ratio between absorbance at 465 and 665 nm (E_4/E_6), and the molar absorptivity at 280 nm (ε_{280}) [9] were obtained.

Fluorescence measurements were obtained using a JASCO FP-6500 fluorescence spectrophotometer. Instrumental parameters were excitation (EX) and emission (EM) slits: 5 nm; response time: 8 s; and scan speed: 240 nm min^{-1}. The EM spectra were obtained by using 254 nm for EX and EM recorded from 280 to 500 nm. Fluorescence intensity value is relative to the instrument conditions at the time of measurement and is a function of source intensity, optical efficiency, and detector efficiency. The sensitivity and stability of the instrument was measured using the Raman band signal intensity (EX, 350 nm; EM, 397 nm). The Raman band intensity was determined prior to each sample and the fluorescence intensities were divided by the Raman intensity to correct for any fluctuations in instrumental conditions. The humification index (HIX) was determined using inner filtering-corrected fluorescence emission spectra, following the equation:

$$HIX = (\Sigma I_{435-480})/(\Sigma I_{300-345}) \quad (1)$$

where I is the fluorescence intensity at each wavelength [35].

2.3.2 Soil respiration

The soil used to test soil respiration was a typical soil from Mediterranean forest, it was sampled from the top layer (0–20 cm) of Torretes Biological Station (Alicante) Southeast Spain (38° 24′ 10″ N; 0° 24′ 54″ W). The soil was manually treated to remove gravel and passed through a 2 mm sieve. The soil is sandy loam, alkaline, calcareous, high in organic matter, and classified as Calcaric Cambisol [12]. The organic materials (QW, QFL, QFH) were added to the soil at 1 g organic material/100 g soil, soil without addition of organic materials was used as a control (CTRL). Soil respiration was measured daily according to Hernández and García [15] in order to determine the mineralisation rate of the organic materials. Respiration rates were measured in hermetically-sealed flasks, in which 30 g of the mix of organic material/soil was kept in the dark at 28 °C and 60% of its water holding capacity for 22 days. The CO_2 emitted was measured by titration with an alkaline solution. Accumulative respiration was calculated by summing the respiration rates per day.

2.4 Statistical analysis

Significant differences ($p < 0.05$) among t reatments were calculated by using one-way analysis of variance (ANOVA) and the Duncan post hoc test. Repeated measures analysis was used for soil respiration. All statistical tests were performed using the statistical package SPSS 15.0 for Windows.

3 Results

3.1 Chemical composition of *Quercus* wood versus *Cetonia* faeces

The comparison of the composition of the laboratory faeces of *C. auratаeformis* (QFL) and the faeces collected in hollows of *Q. rotundifolia* trees in Cabañeros National Park (QFH) with the *Q. rotundifolia* dead wood (QW) showed virtually no change in the O/C and H/C ratios (Table 1). In contrast, the concentration of C, H, O and Ca in QFH was lower than in QFL and QW, while the concentration of Cu was higher in QFH than in QFL and QW (Table 1). The concentration of the other elements analysed (N, P, Mg, K, Fe, Mn, and Zn) and ashes was higher in the faeces than in wood, lastly the C/N ratio in QW was higher than in QFL and QFH, chiefly for faeces collected in hollows (Table 1).

Table 1 Composition of *Quercus rotundifolia* wood (QW), faeces of *Cetonia* reared with *Q. rotundifolia* in laboratory (QFL) and *Cetonia* faeces from *Q. rotundifolia* hollows (QFH).

alt-text: Table 1

	QW	QFL	QFH
C % (w/w)	48.9 ± 0.2 a	48.3 ± 0.2 a	37.6 ± 0.3 b
H % (w/w)	5.62 ± 0.01 a	5.62 ± 0.03 a	4.59 ± 0.05 b
N % (w/w)	0.49 ± 0.01 c	0.83 ± 0.01 b	1.81 ± 0.03 a
O % (w/w)	44.6 ± 0.2 a	41.0 ± 0.2 a	33.8 ± 0.5 b
C/N	99 ± 2 a	58 ± 4 b	22 ± 7 c
O/C	0.91 ± 0.08 a	0.85 ± 0.09 a	0.90 ± 0.08 a
H/C	0.115 ± 0.002 a	0.116 ± 0.006 a	0.122 ± 0.003 a
Ashes % (w/w)	0.32 ± 0.02 c	4.2 ± 0.3 b	22 ± 2 a
P % (w/w)	0.023 ± 0.001 b	0.08 ± 0.02 a	0.07 ± 0.01 a
Ca % (w/w)	2.1 ± 0.1 a	2.27 ± 0.03 a	1.8 ± 0.1 b
Mg % (w/w)	0.14 ± 0.02 c	0.22 ± 0.03 b	0.30 ± 0.04 a
K % (w/w)	0.14 ± 0.01 c	0.35 ± 0.02 b	2.6 ± 0.4 a

Na % (w/w)	0.28 ± 0.08 b	0.46 ± 0.04 a	0.28 ± 0.09 b
Fe (ppm)	326 ± 46 c	1286 ± 25 b	4065 ± 201 a
Mn (ppm)	60 ± 3 c	75 ± 2 b	479 ± 20 a
Cu (ppm)	7 ± 1 b	8.5 ± 0.5 b	21 ± 8 a
Zn (ppm)	35 ± 2 c	47 ± 3 b	242 ± 12 a

Mean values within the same row followed by the same letter indicate the absence of a statistically significant difference between samples ($p < 0.05$).

In the thermal curve of *Q. rotundifolia* wood (QW, Fig. 2), a shoulder at 280 °C and two peaks at 324 °C and 455 °C were observed, which can be assigned to the thermal destruction of hemicellulose, cellulose and lignin, respectively [11,26,31]. The comparison of that thermal curve with the corresponding thermal curve of QFL (Fig. 2) did not show the shoulder assigned to the hemicellulose, instead maintaining the peak about 300 °C. Meanwhile, two peaks at 417 °C and 462 °C appeared in the region corresponding to the lignin. The faeces from hollows showed two broad peaks, one of them at 283 °C, corresponding to decomposition of hemicellulose, and the other at approximately 465 °C, a temperature higher than that usually presented by lignin in wood (QFH, Fig. 2). *Q. rotundifolia* wood had a greater loss of weight in the region of polysaccharides (cellulose and hemicellulose) than in the lignin region. The QFL presented similar losses of weight in both regions, whereas the QFH had a loss of weight higher in the lignin region (Table 2).

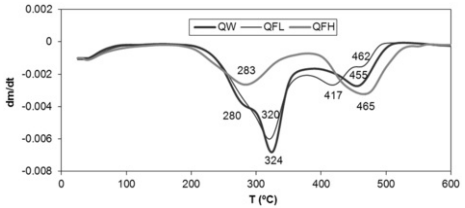

Fig. 2 Thermal curves of *Quercus rotundifolia* wood (QW), faeces of *Cetonia* reared with *Q. rotundifolia* faeces in laboratory (QFL) and *Cetonia* faeces from *Q. rotundifolia* hollows (QFH).

alt-text: Fig. 2

Table 2 Data from different analytical techniques for characterisation applied to the different samples. *Quercus rotundifolia* wood (QW), faeces of *Cetonia* reared with *Q. rotundifolia* in laboratory (QFL) and *Cetonia* faeces from *Q. rotundifolia* hollows (QFH).

alt-text: Table 2

TA†	QW	QFL	QFH
Polysaccharides	49 ± 1 Aa	46 ± 2 Aa	25 ± 1 Bb
Lignin	37 ± 2 Bb	46 ± 1 Aa	37 ± 2 Ab
P/L	1.3 ± 0.1a	0.98 ± 0.02b	0.69 ± 0.4b
FTIR: Relative intensities of the main absorption bands‡			
3	8.3 ± 0.7 a	3.1 ± 0.4 b	absent
5	18 ± 2 b	16.9 ± 0.4 b	23 ± 2 a
6	7.9 ± 0.3 c	9.6 ± 0.2 b	15 ± 1 a
7	9.11 ± 0.07 Cc	10.33 ± 0.07 Cb	12.7 ± 0.4 Ba
8	9.3 ± 0.2 Cc	11.4 ± 0.2 Bb	14.9 ± 0.5 Aa

9	10.03 ± 0.06 Ba	9.32 ± 0.09 Db	absent
10	13.9 ± 0.8 Aa	12.0 ± 0.1 Ab	9 ± 1 Cc
11	–	8.1 ± 0.1 a	5 ± 1 b
12	11.7 ± 0.9 a	8.35 ± 0.06 b	5 ± 1 c
13	8.6 ± 0.6 a	8.3 ± 0.3 a	absent
17	3.4 ± 0.5 a	2.5 ± 0.1 b	4.3 ± 0.5 a b
Ratios§			
I_6/I_3	1.0 ± 0.1 b	3.1 ± 0.5 a	–
I_6/I_9	0.79 ± 0.02 b	1.03 ± 0.01 a	1.1 ± 0.2 a
I_6/I_{13}	0.92 ± 0.09 b	1.15 ± 0.06 a	–
I_6/I_{17}	2.4 ± 0.5 b	3.7 ± 0.2 a	3.6 ± 0.1 a
I_5/I_6	2.2 ± 0.1 a	1.77 ± 0.02 b	1.6 ± 0.1 b
NMR# Regions (ppm)			
0-47 C-alkyl	9	24	18
47-60 N-alkyl/methoxy	10	26	10
60-90 O-alkyl	51	29	45
90-110 O_2-alkyl	10	4	7
CH/Alkyl	7	1	3

†Weight loss (%) at different ranges of temperature and ratio between loss of weight of polysaccharides and lignin.

‡Data are given as percentage of the sum of the heights of the main bans of IR in the region from 1800 to 600 cm^{-1}.

§Ratios between the intensities of bands associated with lignin (5, 6) and bands associated with polysaccharides (3, 9,13,17).

#Relative values (%) of the integrals of the different regions of the spectrum of NMR.

Mean values within the same row followed by the same lowercase letter indicate the absence of a statistically significant difference between samples ($p < 0.05$).

Mean values within the same column followed by the same uppercase letter indicate the absence of a statistically significant difference between parameters ($p < 0.05$).

The FTIR spectra of *Q. rotundifolia* wood and faeces showed a strong absorption band corresponding to the elasticity of the bonds of inter- and intramolecular hydrogen bridges (OH···O) between 3340 and 3350 cm^{-1}, as well as a remarkable band between 2920 and 2925 cm^{-1} due to asymmetric elasticity of CH bonds in aromatic methoxyl groups and methyl and methylene groups of lateral chains [39,40]. Furthermore, in the fingerprint region of the wood (1800–600 cm^{-1}) (Fig. 3) a group of well-defined bands was observed. Some bands, such as bands 5 and 6, were assigned to different lignin groups, whereas bands 7, 8, 10, 12, 14 and 16 were assigned to lignin and polysaccharides, and bands 3, 9, 13 and 17 were assigned to polysaccharides only [34,37,39,40].

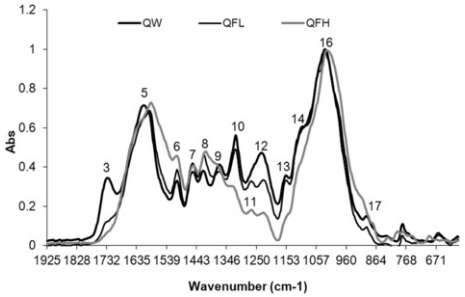

Fig. 3 FTIR spectra of the different samples. *Quercus rotundifolia* wood (QW), faeces of *Cetonia* reared with *Q. rotundifolia* faeces in laboratory (QFL) and *Cetonia* faeces from *Q. rotundifolia* hollows (QFH).

alt-text: Fig. 3

The comparison of FTIR spectra of *C. aurataeformis* larvae faeces (both QFL and QFH) with those of *Q. rotundifolia* wood (Fig. 3), all normalised to the band with the highest intensity (1028 cm^{-1}), showed that the band assigned to hemicellulose (3, Fig. 3) had a low intensity after ingestion. Moreover, it virtually disappeared in the faeces collected in hollows. Band 9, allocated to cellulose and hemicellulose, presented a lower relative intensity in laboratory faeces (QFL) than in wood (QW) (Table 2), and it was absent in the faeces collected in hollows (QFH). An important modification was observed in the region between 1180 cm^{-1} and 1280 cm^{-1} (Fig. 3). Band 11, corresponding to guaiacyl [39], was not present in QW, and band 12, allocated to siringyl and cellulose [39], showed a strong intensity, but both bands were well defined in faeces spectra. The band associated with guaiacyl rings (6, Fig. 3) exhibited a relative intensity higher in the spectra of faeces than in those of wood (Table 2). In addition, the relative intensity of band 5, related to siringyl rings [39], was higher in QFH with respect to wood spectra and lower in QFL (Table 2). Bands 10 and 12 showed a lower relative intensity in faeces spectra than in those of wood, especially for QFH (Fig. 3, Table 2). Comparison of the relative intensity of bands 7, 8, 9 and 10 presents the following order for the different samples: 10 > 9 > 8 = 7 for QW; 10 > 8 > 7 > 9 for QFL; and 8 > 7 > 10 for QFH (Table 2).

In contrast, the ratio between the intensity of band 6, characteristic of lignin [40], and the intensity of the bands allocated to polysaccharides (3, 9, 13 and 17) was greater for faeces than for wood, suggesting a lower concentration of polysaccharides in faeces in comparison to wood. Furthermore, the intensity of bands at 1590 cm^{-1} and 1504 cm^{-1} (I_5/I_6), typical of the ratio between siringyl and guaiacyl structures [34], was lower for faeces than wood (Table 2), showing a preferential decomposition of siringyl versus guaiacyl.

Resonances in the region 60–110 ppm, corresponding to the polysaccharides cellulose, hemicellulose and pectin [1], were predominant in NMR spectra of both wood and faeces. Comparison of relative values of the integrals of the different regions of the NMR spectrum showed that the largest relative resonance corresponded to the O-alkyl region, typical of easily degradable compounds such as cellulose or hemicellulose [17,30,46]. This was observed both in wood and faeces samples. Faecal samples exhibited lower relative resonances in the regions 60–90 ppm and 90–110 ppm, which are allocated to cellulose [47], than the wood sample, especially QFL (Table 2). However, the relative resonance corresponding to C-alkyl (0–47 ppm), associated to aliphatic chains as lipids, cutins and suberins [30], was greater in faeces than in wood. The resonance related to N-alkyl and methoxy (47–60 ppm), allocated to lignin [30], was greater for QFL than in wood, although QFH was not modified (Table 2). In general, the ratio between the resonance of polysaccharides, 60–110 ppm [1], and the resonance of C-alkyl, 0–47 ppm, was lower in faeces than in wood.

3.2 Humification grade of *Cetonia* faeces and their effect on soil respiration

The quantity of soluble organic matter (SOM) in water was larger in QFH (Table 3). Furthermore, SOM from faeces, especially those collected from hollows, had lower carbon concentration and higher nitrogen concentration than did samples of wood (Table 3). The absorption of UV-Vis radiation between 200 and 500 nm of the extracts was as follows: QFH > QW > QFL. Using the extracts of SOM, numerous indices were calculated from UV-Vis radiation and measurement of fluorescence. These indices allow for the determination of the grade of transformation of organic matter (humification process) [5,9,35]. The values for indices E_{ET}/E_{Bz}, E_4/E_6, and ε_{280} were obtained from UV-Vis spectra, while HIX (humification index) was calculated from fluorescence spectroscopy. All indices were higher in faeces (from laboratory and hollows) than in wood (Table 3).

(Table 3: letters and numbers must be located at the same row.) **Table 3** Characteristics of soluble organic matter (SOM) in water. *Quercus rotundifolia* wood (QW), faeces of *Cetonia* reared with *Q. rotundifolia* faeces in laboratory (QFL) and *Cetonia* faeces from *Q. rotundifolia* hollows (QFH).

alt-text: Table 3

Sample	SOM (%)	Elemental Composition			UV-visible parameter			Fluorescence
		C (%)	N (%)	H (%)	E_{Et}/E_{Bz}	E_4/E_6	ε_{280}	HIX

QW	3.6 ± 0.2 b	42.3 ± 0.2 a	1.00 ± 0.02 c	4.32 ± 0.08 a	0.48 ± 0.01 c	8.2 ± 0.5 b	51 ± 1 c	5.1 ± 0.2 c
QFL	3.3 ± 0.4 b	37.6 ± 0.2 b	1.24 ± 0.04 b	4.28 ± 0.03 a	0.56 ± 0.01 a	10.2 ± 0.3 a	56 ± 2 b	12.6 ± 0.1 b
QFH	7.6 ± 0.2 a	33.8 ± 0.2 c	2.08 ± 0.07 a	3.06 ± 0.06 b	0.51 ± 0.002 b	10.5 ± 0.2 a	203 ± 9 a	17 ± 1 a

Mean values within the same column followed by the same letter indicate the absence of a statistically significant difference between samples ($p < 0.05$).

The accumulative respiration per gram of soil increased quickly during the first week of incubation, independently of the addition of the studied samples to the soil (Fig. 4). During incubation, the incorporation of organic materials gave a statically significant rise to a larger accumulative respiration, the QW and the QFH had similar values of respiration while the QFL showed the highest values (Fig. 4).

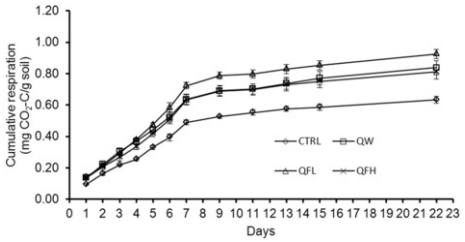

Fig. 4 Accumulative respiration per g soil over 22 days. CTRL: soil without any modification. QW: soil with the addition of *Quercus rotundifolia* wood. QFL: soil with the addition faeces of *Cetonia* reared with *Q. rotundifolia* faeces in laboratory. QFH: soil with th addition of *Cetonia* faeces from *Q. rotundifolia* hollows. Vertical bars indicate standard error (n = 4).

alt-text: Fig. 4

4 Discussion

4.1 Action of *Cetonia aurataeformis* larvae on *Quercus rotundifolia* wood

The analysis of wood and the faeces of larval *C. aurataeformis* showed a higher concentration of minerals, nitrogen and phosphorous in faeces than in *Q. rotundifolia* wood, especially in faeces from hollows (Table 1). These data agree with Micó et al. [31]; who first proved that the faeces of larval *C. aurataeformis* were enriched in minerals, nitrogen and phosphorous after ingestion of different woody substrates (litter, *Betula alba* and *Q. pyrenaica* wood). The higher concentration in N and P in faeces than in wood may be due partly to the digestion of polysaccharides which would reduce carbon relative to nitrogen and phosphorous, thus increasing concentration in N and P. That increase in N could be also due to the possibility of N_2 fixation in the gut of *C. aurataeformis* by endosymbiont bacteria. Nitrogen fixation appears to be widespread among wood-feeding insects, with evidence in at least 60 species of termites, beetles and a wood wasp [48]. Moreover, according to Ulyshen [48]; some studies show elevated rates of nitrogen fixation in the faeces-filled tunnels of termites, ants, passalid beetles and other insects, suggesting that faeces may be a preferred substrate for N_2-fixing bacteria. This situation could help to explain the higher N concentration in QFH than QFL found in this article, as faeces from the laboratory are removed often for analysis, while faeces from hollows remain in place for months or even years, allowing for N_2-fixing bacteria.

The thermal analysis of *Q. rotundifolia* wood showed a loss of weight greater in the region of polysaccharides than in the region of lignin, whereas the QFL had a similar loss of weight in both regions. Micó et al. [31] also found similar losses of weight in the regions of lignin and polysaccharides during the thermal analysis of faeces of larval *C. aurataeformis* fed in a laboratory with *Q. pyrenaica* and litter from Mediterranean brushwood. In contrast, in nature the faeces from hollows suffered a loss of weight larger in the lignin region than in the polysaccharides region (Table 2). These data could indicate a higher concentration of polysaccharides in the wood than in faeces. Furthermore, the faeces from the laboratory (QFL) had, proportionally, a higher concentration of polysaccharides than faeces obtained in hollows (QFH), suggesting a preferential consumption of polysaccharides over lignin by the larvae. That fact, along with the disappearance of the shoulder at 280 °C in the thermal curves corresponding to the faeces (Fig. 2), could suggest the digestion of polysaccharides by the larvae.

The decomposition of polysaccharides by larval *C. aurataeformis* is also supported by the results of IR spectroscopy, which showed that the intensity of the band for lignin (6) and the ratios between the intensities of the band for lignin and bands allocated to polysaccharides (I_6/I_3, I_6/I_9, I_6/I_{13}, I_6/I_{17}), were higher in faeces than in wood, whereas the bands corresponding to polysaccharides (3, 9, 13 and 17) manifested lower relative intensities in faeces than in wood (Table 2). Moreover, band 3, associated with hemicellulose, and band 9, corresponding to cellulose and hemicellulose, were not only decreased but were, in fact, not found in the faeces from hollows.

The digestion of polysaccharides by larval *C. aurataeformis* would also agree with the presence of band 11 in the faeces, related to guaiacyl [39]. This is likely related to the disappearance of the polysaccharides in band 12,

associated with siringyl and cellulose [37,39], which showed decreased relative intensity in faeces. The digestion of polysaccharides is in concordance also with the increase of the relative intensity of band 8 versus bands 7, 9 and 10 in faeces, especially of hollows (QFH), versus wood (Table 2). The results obtained with IR spectroscopy agree with studies of degradation of wood by other authors [7,10,37], which showed that the degradation of the polysaccharides in wood produced a residue richer in lignin.

Similar results of thermal analysis and FTIR were obtained by Micó et al. [31] after ingestion of different materials (*Betula alba*, *Q. pyrenaica* and litter from Mediterranean brushwood) by larval *C. aurataeformis* in the laboratory. The thermal analysis showed a disappearance of the bands associated with polysaccharides (3, 9 and 13) and a higher reduction of the relative intensity of band 12 in the IR spectrum of faeces from hollows, which would agree with a lower concentration of polysaccharides in faeces from hollows versus faeces from the laboratory (Table 2).

The NMR data also showed a decrease in polysaccharides concentration after ingestion of *Q. rotundifolia* by larval *C. aurataeformis*. Thus, the relative resonances of the regions 60–90 ppm, characteristic of compounds easily degradable as cellulose and hemicellulose, and 90–110 ppm, allocated to cellulose, were less in the faeces that in wood. In contrast, the relative resonance corresponding to structures that are more stable (lipids, cutins and suberins, 0–47 ppm) was higher in faeces than in wood (Table 2).

In this way, the comparison of *Quercus* wood with faeces (QFL and QFH) clearly showed the preferential decomposition of polysaccharides versus lignin, which is likely due to the selective digestion of peptide and polysaccharide components over aromatic components, as found in the humivorous larvae of *Pachnoda ephippiate* (Coleoptera: Scarabaeidae) [23,25]. Martínez-Sabater et al. [30] also found a decrease in polysaccharides concentration and an increase in the concentration of molecules resistant to biodegradation (e.g. suberin and cutin) during compost processing of organic wastes.

The preferential decomposition of polysaccharides by wood-feeding insects has been correlated with enzymatic degradation caused by endosymbiotic cellulolytic agents in the guts of wood-feeders, and endogenous cellulases [49,52].

The ingestion of *Q. rotundifolia* wood by larval *C. aurataeformis* also generates a decline in the ratio of C/N (Table 1). The lower results of the C/N ratio in faeces than in wood (Table 1) could be due to both the digestion of polysaccharides and the ability of wood-feeding insects to fix nitrogen [25,48]. Considering that the faeces from the laboratory had a higher ratio of C/N and higher concentration of carbohydrates than the faeces from hollows, according to the thermal analysis and FTIR spectroscopy (Tables 1 and 3), it could be assumed that the faeces from hollows have a level of transformation larger than the ones from the laboratory. The transformation of the faeces from hollows could be due to the action of different microorganisms (such as bacteria and fungi). While the faeces from the laboratory are removed often for analysis, faeces from hollows stay in the same location for months to years, allowing their modification by microfauna. Because of this, Ulyshen [48] suggests that the faeces of wood-feeding organisms are a preferred substrate for N_2-fixing bacteria.

In contrast, Li and Brune [25] found that fresh faecal pellets of humivorous larval *Pachnoda ephippiate* contain a great quantity of ammonium that could be used by organisms, so the ammonification process for assimilation of nitrogen would be not necessary in that case. Therefore, it could be hypothesised that the faeces of *C. aurataeformis* contain large quantities of ammonium which facilitate the utilisation of that faeces by the microorganisms living in the tree hollow.

The distribution of thermogravimetric curve peaks (Fig. 2) could indicate that the fraction of polysaccharides in the faeces from hollows (QFH) is less stable than the corresponding faeces from the laboratory (QFL), which presents the same stability as that of the cellulose of wood. The low stability of the fraction of polysaccharides in QFH would be due to the higher transformation of *C. aurataeformis* faeces by the biota living in the hollow tree. According to Ulyshen [49]; wood is only partially decomposed after a single passage through an invertebrate and some taxa are less efficient assimilators than others (e.g. beetle larvae assimilate consumed wood less efficiently than termites). Fresh faeces are then colonised and further digested by microbes before reingestion by invertebrates of the same or different species.

In the QFH samples, the lignin region showed a fraction with a higher stability than the lignin of wood, while the QFL had structures with lower and higher stability than the initial (Fig. 2). The change of the lignin can be related to the decrease of ratio of the relative intensities of bands 5 and 6 of the FTIR spectrum (I_5/I_6, Table 2), which suggests a decrease of the rings of siringyl with respect to rings of guaiacyl for QFL and QFH samples. Different studies have presented a preferential decomposition of siringyl units in comparison to guaiacyl units during the process of transformation of different woods [47]. Vane et al. [51] consider that the decrease of the ratio of I_5/I_6 is an indicator of biodegradation of the lignin. Those data could be due to a minor modification of the organic materials in the faeces from the laboratory with preferential digestion by larvae of the fractions of polysaccharides that are less stable (hemicellulose), and an important change of the lignin may generate two fractions of different stability [31]. The faeces collected in hollows of *Q. rotundifolia* trees showed a deep modification, originating two groups of structures, one with low stability and the other with high stability, as occurs in the composting process and humification [2,9,30].

Selective digestion of less recalcitrant components will automatically increase the stability of the residual organic matter [13,23]. Li and Brune [23] found that humivorous beetle larvae *Pachnoda ephippiata* selectively digest the peptide and polysaccharide components of humic substances, whereas the aromatic components of humic substances are not an important source of nutrients and energy. Those results are in accordance with the increase of the temperature localised at the lignin region of the thermal curves corresponding to *C. aurataeformis* faeces versus the wood substrate (Fig. 2).

4.2 Humification grade of faeces and their effect on soil respiration

SOM in water is the most labile fraction of organic matter, it is also the most reactive in the soil, and it is an intermediate phase between the initial biotic residue and the humic substances which are the final product in the soil [35,36]. The process of humification generates organic materials with high total exchangeable acidity, high concentration of carboxylic groups, moieties with nitrogen [35], and an increase of the aromatic character of the organic molecules, shown by the presence of aromatic rings and condensed polyaromatic structures [5,9]. Of the three substrates investigated in this study, the faeces from hollows had the largest fraction of soluble material in water, while the *Q. rotundifolia* wood and the faeces from the laboratory had similar fractions of soluble material (Table 3). The composition of SOM was different for the three materials, with the order of the carbon concentration as follows: QW > QFL > QFH, whereas the order of the nitrogen concentration was the inverse (Table 3). This could be due to a higher level of modification or humification of the SOM from the faeces collected inside hollows and a lower level in the faeces from the laboratory, explained in Section 4.4. The SOM of *Q. rotundifolia* would be organic matter richer in carbon and non-humified.

Indices obtained from UV-Vis spectroscopy and fluorescence have been used to determine the level of humification in past studies, differentiating the humic substances from non-humic materials [5,9,14,35]. The correction of Ohno [35] on the humification index (HIX) obtained from fluorescence data allows for comparison of the level of humification of samples with the same origin. A higher HIX value corresponds to a higher level of humification, since a high level of humification produces a high level of aromaticity and an increase in the emission of fluorescence of long wavelengths (435–480 nm) versus short wavelengths (300–345 nm). According to Ohno [35]; the HIX values obtained in this study indicate that the QFH samples have a higher humification than the QFL (Table 3), which is in concordance with the larger transformation of QFH compared to the QFL (see Section 4.4).

The ratio of absorbances at 465 and 665 nm (E_4/E_6) is a typical index obtained from UV-Vis spectroscopy to determine the aromaticity, and a high value of E_4/E_6 can be inversely correlated to the aromaticity. However, E_4/E_6 ratios have shown to be better correlated with molecular size, O/C and C/N atom ratios, carboxyl concentration, and total acidity than with aromaticity and, therefore, may be better suited as a general tracer of humification [14]. The E_4/E_6 index was higher for the faeces than for the wood, although differences between the faeces from the laboratory and from nature were not significant (Table 3). Fuentes et al. [9] also found higher values of E_4/E_6 for composted materials than for original materials, which would allow differentiation among materials that have been processed, although the level of transformation could not be calculated.

The molar absorptivity at 280 nm based on a mole of organic carbon (ε_{280}) has been used as indicator of aromaticity because of the difficulty of determining aromaticity from soluble organic matter using E_4/E_6 [9]. The ε_{280} values obtained in this study (Table 3) suggest the following order in the level of aromaticity for the different samples: QFH > QFL > QW. This agrees with the high and positive correlation between ε_{280} and the level of aromaticity established by different authors which have worked with aquatic humic substances [9], and with the preferred ingestion of polysaccharides over lignin by larval *C. aurataeformis*, established in Section 4.1.

The index E_{ET}/E_{Bz} is the ratio of absorbances at 253 and 203 nm, corresponding to the electron-transfer band (ET) and benzenoid band (Bz) of benzene UV light absorption, respectively. Low ratios of E_{ET}/E_{Bz} are associated with scarce substitution of aromatic rings or with the substitution of aliphatic groups, whereas high E_{ET}/E_{Bz} is indicative of the presence of moieties with oxygen in the aromatic ring (hydroxyl, carbonyl, carboxyl and ester) [9]. The higher values of E_{ET}/E_{Bz} in faeces than in wood (Table 3) would suggest a larger level of substitution in the aromatic structures with moieties that contain oxygen. This behaviour agrees with Fuentes et al. [9]; who found an increase of the ratio of E_{ET}/E_{Bz} after composting of different wastes (ovine manure, mixture of animal manure, olive wastes, grape wastes, and domestic wastes). The different spectroscopic indices lead to the conclusion that SOM from of *C. aurataeformis* faeces have a higher concentration of aromatic structures with a higher grade of substitution than wood. Furthermore, the proportion of moieties of the aromatic rings of each kind of faeces was different.

The analysis of SOM from the three materials evaluated (Table 3) could indicate that the increase of the grade of transformation/humification of the material produces solubilisation of a great quantity of organic compounds with high nitrogen concentration. This would facilitate the development and activity of biota in the hollow trees, particularly Sánchez-Galván et al. [43] showed that saproxylic syrphid larvae growing in a substrate enriched with cetonid larval faeces had better development and fitness.

Cobb et al. [6] found that organic nutrient inputs in the form of wood-feeding bettles faeces increased mineral soil microbial respiration rates which is consistent with our results (Fig. 4). This can be due to the utilisation of organic compounds that are easily available, which are often soluble in water. The presence of substrates that are easily available also stimulates the decomposition of more recalcitrant materials [44]. All of this agrees with our finding that the highest levels of accumulative respiration were found in soils where organic materials were incorporated (Fig. 4). Although the concentration of SOM for QW and QFL were of the same order (Table 3), the greater accumulative respiration found in the faeces from the laboratory could be due to a percentage of its lignin fraction (35%) being less stable than the lignin of QW (Fig. 2). This could lead to lower growth of microorganisms in QW, which would use their energy in the synthesis of specific enzymes for the decomposition of materials with low solubility [44].

The removal of the soluble fraction of plant residues produces an initial reduction of accumulative respiration with a subsequent increase until values near to the ones of the wastes without removal of soluble compounds are reached [44]. Moreover, those authors determined that there was a higher proportion of microorganisms of slow growth (K-strategists) in the extracted residue compared to the microorganisms of fast growth (r-strategists) found in original residue. In contrast, the faeces from the hollows had the highest percentage of SOM among the three organic materials, although it had lower polysaccharides concentration than lignin concentration (Table 2). The lignin fraction of QFH was also more stable that the ones of QW and QFL (Fig. 2), which could mean that the accumulative respiration of QW and QFH were similar (Fig. 4).

Past research into the effects of the faeces of different invertebrates which feed on plant litter usually show an increase in microbial respiration after defecation, after which microbial respiration decreases and the respiration in the old faeces becomes lower than that of an intact original leaf [19]. This agrees with the lower accumulative respiration obtained in this study for the faeces from hollows with respect to faeces from the laboratory, and also agrees with the similar values found for the accumulative respiration of QW and QFH (Fig. 4).

In conclusion, composition and grade of humification of the SOM fraction of *C. aurataeformis* faeces and their effect on soil respiration suggest that the incorporation of *C. aurataeformis* faeces into soil involves an input of organic matter with a good level of humification and with medium-high availability for soil microorganisms, which are primarily responsible for growth and plant nutrition in soils. In consequence, the action of this cetonid accelerates the carbon and nitrogen cycle in saproxylic environments.

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Highlights

- Larval *Cetonia aurataeformis* produce a residue with higher content of N and P.
- Larval *Cetonia aurataeformis* decompose the polysaccharides in *Quercus* wood.
- The stability of lignin increases with the time that faeces are in *Quercus* hollows.
- The humification of Cetonid faeces increases with the time in *Quercus* hollows.

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