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DEGRADATION OF LEAF LITTER PHENOLICS BY AQUATIC AND TERRESTRIAL ISOPODS

MARTIN ZIMMER,[1,*] RICARDO OLIVEIRA,[2] ELSA RODRIGUES,[2] and MANUEL A. S. GRAÇA[2]

[1]*Zoologisches Institut: Limnologie, Christian-Albrechts-Universität zu Kiel, Olshausenstr. 40, D-24098 Kiel, Germany*

[2]*Departamento de Zoologia and IMAR, Universidade de Coimbra, 3004-517 Coimbra, Portugal*

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Abstract—To investigate species-specific decomposition rates of litter from native (*Quercus faginea*) and introduced (*Eucalyptus globulus*) tree species in Portugal, we monitored changes in the phenolic signature of leaf litter during decomposition as mediated by an aquatic, *Proasellus coxalis* (Isopoda: Asellota), and two terrestrial, *Porcellio dispar* and *Eluma caelatum* (Isopoda: Oniscidea), detritivores. Although the litter of *Eucalyptus* and *Quercus* did not differ in overall protein precipitation capacity, we detected differences in terms of contents of particular phenolic compounds and phenol oxidation products. Accordingly, we observed food-specific consumption rates in *Proasellus*, but not in the terrestrial isopods. *Proasellus* digested *Eucalyptus* at significantly higher rates than *Quercus*, whereas the opposite was the case for *Eluma*, and *Porcellio* digested both litter types equally well. Despite slight differences in detail, effects of *Proasellus* on changes in the signature of litter phenolics were similar for both litter types, whereas terrestrial isopods—*Porcellio* and *Eluma*, although they differed from each other—digestively degraded phenolic compounds in *Eucalyptus* and *Quercus* litter, respectively, in different ways. Overall, however, degradation of litter phenolics was similarly effective on both litter types. From these data, we conclude that decomposition of *Eucalyptus* litter does not proceed more slowly than of litter from native Portuguese trees.

Key Words—Animal–microbe interactions, decomposition, *Eucalyptus*, Isopoda, neophytes, phenolics, *Quercus*, tannin degradation.

* To whom correspondence should be addressed. E-mail: mzimmer@zoologie.uni-kiel.de

INTRODUCTION

Eucalyptus globulus Labill., native in Australia, has been planted during the last 170 yr in southern European countries, South America, and other parts of the world. In Portugal, *Eucalyptus* plantations nowadays occupy more than 20% of the forested area (Canhoto et al., 2002). Several studies demonstrated that *Eucalyptus* plantations affect invertebrate assemblages in soils (e.g., Pinto et al., 1997; Sousa et al., 1997) and freshwaters (e.g., Abelho and Graça, 1996; Graça et al., 2002). In freshwaters, invertebrate shredders may exert an important contribution to the decomposition of leaves entering streams (Graça, 2001); however, invertebrate shredders seem to have difficulties feeding on *E. globulus* leaves (Graça et al., 2002). In soil, terrestrial isopods apparently have the same difficulty using *E. globulus* litter (Sousa, personal communication). *Eucalyptus* litter is, therefore, considered a low-quality food for both freshwater (Mellilo et al., 1982; Campbell and Fuchshuber, 1995) and soil (Sousa, personal communication) detritivores. Such effects may be due to chemical and physical defense mechanisms in leaves. Canhoto and Graça (1999) showed that eucalyptus oils inhibit fungal growth and invertebrate feeding, and a thick cuticle retards microbial colonization of senescent leaves.

Another chemical defense of *E. globulus* leaves is polyphenolics. Canhoto and Graça (1996) observed a strong negative correlation between the phenol content of different native litter types and *Eucalyptus* litter and decomposition rates in a stream, whereas Canhoto and Graça (1999) showed that polyphenolics from eucalypt leaves decrease feeding by detritivores. Thus, effects of phenolics on detritivores may be one reason for the low decomposability of *Eucalyptus* litter. Overall, however, *Eucalyptus* litter decomposed at almost two times higher rates than the Portuguese oak, *Quercus faginea* Lam., the latter containing more than twice as much phenolics than the former but about the same amount of nitrogen (Canhoto et al., 2002). In the present study, we used leaf litter of the native Portuguese oak, *Q. faginea*, and the introduced *E. globulus* (both henceforth mentioned generically) to study in detail changes in the content of specific phenolic compounds during decomposition as mediated by aquatic and terrestrial detritivores.

Both terrestrial Oniscidea and freshwater Asellota had ancestors in the marine environment (that may have been closely related; Zimmer and Bartholmé, 2003). It has recently been discussed to what extent the ability to tolerate or even digest phenolic food compounds may have been important in the evolutionary step to utilizing terrestrial food sources (Zimmer et al., 2002a,b); despite their aquatic lifestyle, freshwater isopods mostly consume leaf litter of terrestrial origin (cf. Zimmer and Bartholmé, 2003) just like their terrestrial relatives do (cf. Zimmer, 2003). We were, thus, interested in whether representatives of different phylogenetic histories that are similar with respect to

available food sources are also similar in their digestive capabilities. Hence, we chose two terrestrial and one freshwater isopods as common Portuguese models for evolutionary ecological studies on digestive capabilities and detritivore–microbe interactions and their consequences for decomposition processes, in particular the degradation of phenolic leaf litter compounds.

METHODS AND MATERIALS

Leaf Litter and Detritivores. We collected leaf litter in spring 2002, when isopods were active in the field and started reproducing, in the Mata Nacional do Choupal, Coimbra (40°12′33″N, 8°90′27″W), Central Portugal. At this time of the year, with high feeding rates by isopods, *Quercus* litter had been lying on the ground for about 1–3 mo, whereas *Eucalyptus* litter was estimated to be up to 6 mo old. Thus, our choice of litter reflected the natural conditions detritivores face during their most active feeding period; many, but not all, species-specific compounds of the litter will have been leached at that stage. We selected leaves that did, upon visual inspection, not show heavy decomposition. In the laboratory, leaves were air-dried in the dark at room temperature and stored dry until needed to avoid further loss of phenolics through leaching or photoautoxidation. We did not test for differences in phenolic signatures of leaf litter before and after storage because we were interested in relative effects of detritivore activity on phenolic litter compounds during decomposition.

Terrestrial isopods, *Porcellio dispar* Verhoeff 1901 (Oniscidea: Porcellionidae) and *Eluma caelatum* [Miers 1877] (Oniscidea: Armadillidiidae), were collected in the Mata Nacional do Choupal by browsing through mixed leaf litter layers in spring 2002. In the laboratory, they were housed in plastic boxes with moist sand (16°C) feeding exclusively on *Quercus* or *Eucalyptus* for at least 5 d. *Proasellus coxalis* [Dollfus 1892] (Asellota: Asellidae) were collected in a small creek, Rio Pavia in Fail (40°36′17″N, 7°58′44″W), near Viseu, northeast of Coimbra, which belongs to the Mondego's drainage basin. In the laboratory, they were housed in plastic boxes with aerated water of the same origin (16°C) feeding exclusively on *Quercus* or *Eucalyptus* for at least 5 d.

Experimental Setup. During experiments ($N = 9$, each), groups of three (terrestrial) or five (aquatic) isopods were kept in small petri dishes, the lids of which were lined with moist filter paper to maintain high humidity or that were filled with filtered creek water, respectively. Feces were collected twice a day to minimize both coprophagy and postdigestive changes in phenolic compounds by picking up fecal pellets with forceps or by filtering the water from the petri dishes after removal of isopods and litter, respectively. Afterwards, isopods

were placed in fresh petri dishes with filter paper-lined lids or filtered creek water, respectively. Feces were immediately stored at −20°C until being used for HPLC analysis.

Single air-dried leaves, randomly chosen from the pool of hand-selected litter (see above), were cut in pieces, two of which were weighed and then placed in either isopod assays or isopod-free controls. Another piece of that same leaf was used to determine the initial phenolic signature of individual leaves as described below. An additional piece of each leaf was weighed, then oven-dried (60°C, 24 hr), and weighed again to obtain a factor to estimate the initial dry mass of litter (Zimmer and Huryn, in press). After the experiment, lasting for 5 d, litter remnants were weighed fresh to calculate mass loss. Litter obtained from parallel experiments that were not used for HPLC analysis was used to determine a factor for the estimation of final dry mass of litter (oven drying at 60°C for 24 hr) as described above.

Consumption rates [mg (mg d)$^{-1}$] were determined based on litter mass loss and mean isopod dry mass. Digestibility (%) was determined as percent of ingested litter that was not egested as feces (Zimmer and Huryn, in press). Direct effects of detritivores on litter chemistry through digestion were quantified by comparing the phenolic signature of litter prior to the experiment with that of isopod feces. Indirect effects of detritivores on litter chemistry (e.g., through mediating microbial decomposition; see Zimmer et al., 2002b, 2004) were estimated by comparing the phenolic signature of litter prior to the experiment with that of unconsumed litter remnants after the experiment. Such indirect effects could have occurred either through selective consumption of litter or through direct influences of detritivores (e.g., fecal amount and composition, mucus, urine, molt deposition, and so on) on type and abundance of microbiota (Zimmer et al., 2002b, 2004).

Leaf Litter Phenolics. For extraction of phenolics, 45–55 mg (dry mass) of leaf litter or 3–10 mg of feces were homogenized in 2 ml 50% aqueous methanol (cf. Van Alstyne, 1995) and shaken for 3 hr at 16°C. After centrifugation, the solvent was replaced by 2 ml 50% methanol, and samples were extracted overnight at 16°C. After centrifugation (10,000 × *g*, 10 min), supernatants were pooled and either used for the determination of protein precipitation capacity or for HPLC analysis after filtering (0.45 μm).

The capability of phenolics to precipitate proteins was measured using the radial diffusion assay (Hagerman, 1987). Portions of 9.5 ml of agarose (1%) containing 0.1% of bovine serum albumin (BSA) were dispensed in 8.5-cm diam petri dishes. Polyphenolics were extracted from 50 mg of leaf powder as described above. Aliquots of the supernatant (36 μl) were inoculated into 4-mm diam wells punched out from the agar plate. A protein precipitation ring was allowed to develop for 3 d at 16°C. The precipitation area was measured and compared to a standard curve using tannic acid (Merck; Ref.: 1.00773.0250;

Lot K18722673703), and the results were expressed in terms of "tannic acid equivalents." We are aware that different phenolic compounds will have different protein precipitation capacities, but because we could not determine most of the compounds, we refrained from using different standards.

For the detection of specific phenolic compounds in litter and feces extracts, we performed RP-HPLC using a Brownlee Column (Applied Biosystems) SPHERI-5 RP-18 (5 μm) 250 × 4.6 (OD-5A) with a NewGuard RP-18 (7 μm) 15 × 3.2 guard column. Peaks were detected at 280 nm. In preruns, no peaks were detected after 45 min; thus, we chose a run time of 45 min. According to previous studies (Scalbert et al., 1988; Streit and Fengel, 1994) and our own preexperiments, we chose a system of two eluants [A, 950:49:1, water/methanol/phosphoric acid (85%); B, 999:1, methanol/phosphoric acid (85%)] with a gradient of 0–5% B during the first 15 min and a subsequent gradient of 5–90% B during 15 min at a flow rate of 1 ml min^{-1} at 20°C.

Besides other phenolic compounds, tannic acid (mainly *penta*-galloylglucose), gallic acid, catechin, and gallocatechin have been isolated from *Quercus* leaves (e.g., Scalbert et al., 1988; Tanaka et al., 1995). We used these compounds (Sigma) as standards for HPLC in concentrations of 0.1, 0.5, and 1.0 mg ml^{-1}. Because tannic acid, rutin, quercetin, and catechol have, besides others, been determined in extracts of *Eucalyptus* leaves (e.g., Conde et al., 1997; Sasikumar et al., 2002), we used these compounds (Sigma) as standards in HPLC (0.1, 0.5, and 1.0 mg ml^{-1}). To detect oxidation products of these model phenolics in experimental extracts, we forced phenol oxidation in standards by both vigorously shaking these solutions for 60 min at RT and adding NaOH solution (32%, pH 12; 1 ml 100 ml^{-1}). After the generation of brown coloration, we determined the elution time of phenol-specific oxidation products by HPLC as described above.

Even with the slowly changing conditions during min 0–15 (see above), we could not unambiguously identify every single peak derived from a complex mixture of litter phenolics and their derivates (see Table 1). Difficulties in separating phenolic compounds by RP-HPLC with water–methanol gradients have previously been reported in flowers by Van de Casteele et al. (1983), in leaves by Koupai-Abyazani et al. (1992), and in wood by Streit and Fengel (1994). We, thus, refrain from assigning phenolic standards to particular HPLC peaks, but refer to phenolic compounds by the name of peaks in order of their elution.

From the areas (units ml^{-1}) of particular peaks as revealed by HPLC analysis of pre- and postexperimental litter and feces, we approximated changes in the content of particular phenolic compounds (%) in isopod-free controls, through isopod-mediated microbial activity, and through isopod feeding and digestion. Further, we estimated litter decomposition in terms of mass loss by calculating consumption rates of detritivores as described in Zimmer and Huryn

TABLE 1. ASSIGNMENT OF STANDARD PHENOLIC COMPOUNDS[a] TO HPLC PEAKS

	C	D	E	F	G	H	M	O	P	R	U
GA					X						
TA	X	X	X	X							
CAT	X										
CATol						X					
gCAT	X										
oxGA		X									
oxTA							X	X			
oxCAT									X		
oxCATol		X									
oxgCAT										X	X

[a] GA, gallic acic; TA, tannic acid; CAT, catechin; CATol, catechol; gCAT, gallocatechin; ox-, oxidation product of -. Other standard compounds could not be assigned to HPLC peaks from experimental samples.

(in press). Because most of our data deviated from normal distribution, we chose median ± median absolute deviation for graphical presentation, and nonparametric tests were used for statistical analysis. Comparison of different treatments (leaf litter or detritivore species) was performed through Mann–Whitney *U*-tests. Differences between initial values and values after experimental treatment were tested with Wilcoxon sign tests.

RESULTS

General Patterns. Although *Quercus* and *Eucalyptus* litter did not differ from each other quantitatively in their initial protein precipitation capacity (radial diffusion assay: *Eucalyptus*, 24 ± 4 mg TA equivalents g^{-1}; *Quercus*, 18 ± 8 mg g^{-1}; $P > 0.7$), they differed qualitatively in terms of contents of different phenolic species and their oxidation products (Figure 1).

While microbial degradation (in animal-free assays) of *Quercus* leaves was not detectable in the terrestrial environment, litter lost 2 ± 1 μg $(mg\ d)^{-1}$ in the aquatic environment ($P < 0.05$), presumably because of microbial degradation and leaching. After correction of data for these control values, *Porcellio* [4 ± 2 μg $(mg\ d)^{-1}$], *Eluma* [7 ± 3 μg $(mg\ d)^{-1}$], and *Proasellus* [3 ± 2 μg $(mg\ d)^{-1}$] did not differ from each other with respect to consumption rates ($P > 0.3$). Digestibility of *Quercus* litter was similar in *Porcellio* (33 ± 17%) and *Proasellus* (31 ± 24%), but significantly higher ($P < 0.05$) in *Eluma* (62 ± 22%).

Microbial degradation and leaching (in animal-free assays) of *Eucalyptus* resulted in litter mass loss of 4 ± 1 μg $(mg\ d)^{-1}$ in the terrestrial environment and 11 ± 9 μg $(mg\ d)^{-1}$ in the aquatic environment ($P < 0.01$). After correction

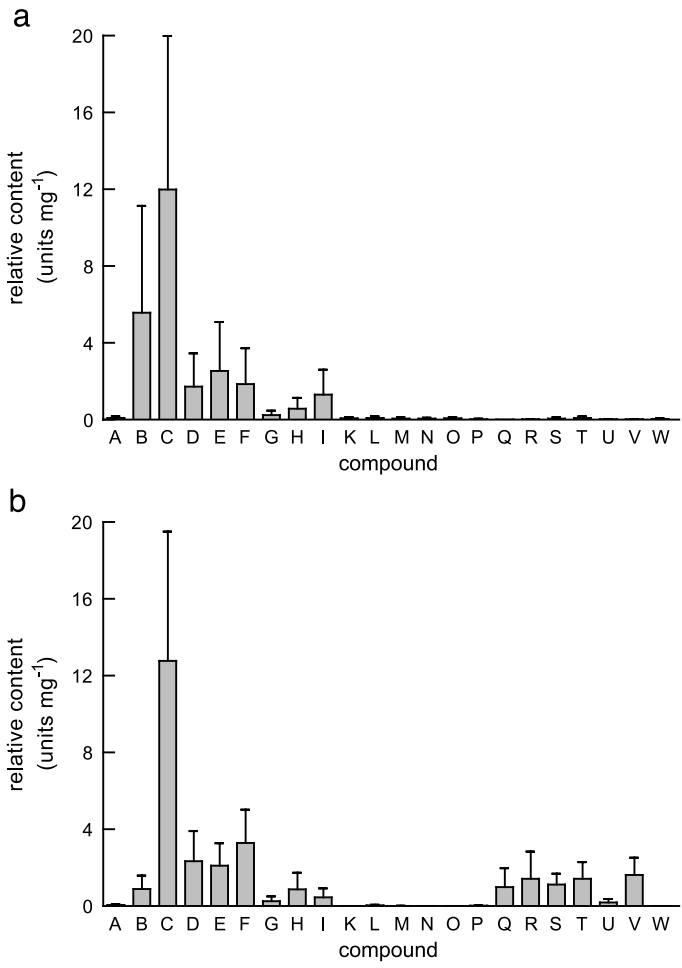

FIG. 1. Phenolic composition of leaf litter derived from *Quercus* (a) and *Eucalyptus* (b). Data are median ± median absolute deviation ($N = 9$).

of data for control values, *Porcellio* [5 ± 3 μg (mg d)$^{-1}$] and *Eluma* [3 ± 2 μg (mg d)$^{-1}$] did not differ from each other with respect to consumption rates ($P > 0.4$), whereas *Proasellus* [30 ± 20 μg (mg d)$^{-1}$] consumed about 10 times more than the terrestrial isopods. Thus, while the terrestrial isopods consumed about the same amounts of *Quercus* and *Eucalyptus*, *Proasellus* ingested significantly more *Eucalyptus* than *Quercus* ($P < 0.01$). Digestibility of *Eucalyptus* litter was similar in *Porcellio* (33 ± 13%) and *Eluma* (26 ± 20%), but significantly higher ($P < 0.05$) in *Proasellus* (71 ± 18%). Thus, *Porcellio* di-

gested both litter types equally well, whereas *Eluma* was significantly ($P < 0.01$) more efficient in digesting *Quercus*, and *Proasellus* digested *Eucalyptus* at significantly ($P < 0.01$) higher rates than *Quercus*.

According to these results, *Proasellus* ingested larger amounts of *Eucalyptus* than *Quercus*. Consumption of *Eucalyptus* differed between consumers, with *Proasellus* consuming the largest amounts, but *Quercus* was ingested in similar rates by all consumers. Accordingly, digestibility of *Eucalyptus* by *Proasellus* was higher than that of *Quercus*. By contrast, although *Eluma*, too, exhibited higher digestibility of *Eucalyptus* than *Porcellio*, the terrestrial species digested both litter types equally well.

General Changes in Phenolic Signatures

Quercus. In the aquatic environment, only compounds B, C, E, I, P, and W were not completely or at least mostly leached, degraded, or transformed in animal-free controls (Figure 2a); compounds E, P, and W were obtained in higher concentration after the experiment than before ($P < 0.05$). With isopods present, compounds C and I were microbially degraded or transformed in the leaf litter that was not consumed by isopods (Figure 3a); microbially processed litter was enriched in compounds N, P, U, and V ($P < 0.01$). On average, compounds B and I were digested completely by litter-feeding isopods (Figure 4a), whereas compounds E, F, H, M, P, T, and V were concentrated higher in feces than in the litter ($P < 0.01$).

In the terrestrial environment, only a few compounds were lost through leaching, degradation, or transformation in animal-free controls (Figure 5a); compounds D, P, and U were obtained in higher concentration after the experiment than before ($P < 0.001$). With *Porcellio* present, compounds I, V, and W were microbially degraded, and compounds E, M, and P became significantly reduced, probably through microbial activity, in the leaf litter that was not consumed by isopods (Figure 6a); microbially processed litter was enriched in compounds L and O ($P < 0.05$). With *Eluma* present, compounds D, E, F, O, and T were microbially degraded (Figure 7a), whereas compounds M, P, U, and V became enriched in the litter ($P < 0.001$). On average, compounds C, U, and V were digested completely in isopod guts, and about 90 and 50% of compounds E and I, respectively, was degraded digestively by *Porcellio* (Figure 8a), whereas compounds D, F, G, H, M, P, R, S, and T were concentrated higher in feces than in the litter ($P < 0.01$). *Eluma* digested all of the remaining compounds B, G, H, I, O, T, and V and reduced compound D by about 85% (Figure 9a), but led to a relative increase of compounds E, M, P, Q, R, U, and W ($P < 0.001$).

Eucalyptus. In the aquatic environment, only compounds B, C, E, H, P, U, and W were not completely or at least heavily leached or degraded in animal-free controls (Figure 2b); compounds H, P, and U were obtained in higher

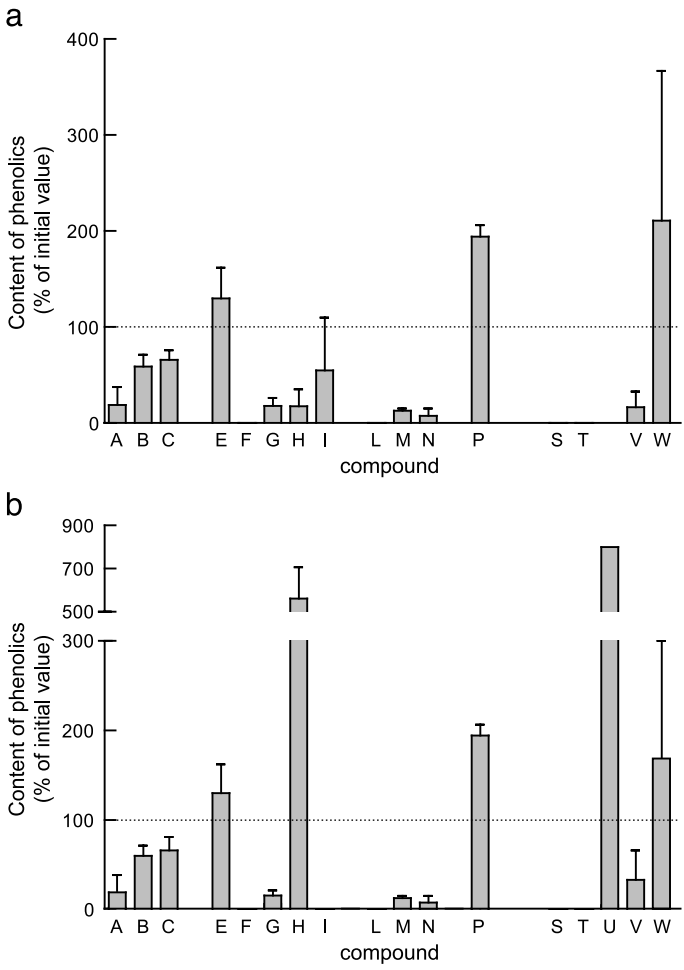

FIG. 2. Change in content of phenolic compounds in leaf litter derived from *Quercus* (a) and *Eucalyptus* (b) due to leaching and microbial processing within 5 d in aquatic animal-free control assays as compared with initial values of the litter (100%). Data are median ± median absolute deviation ($N = 9$). Only those peaks are listed along the *X*-axis that were found in the samples studied here.

concentration after the experiment than before ($P < 0.001$). With isopods present, compound A was microbially degraded or transformed in the leaf litter that was not consumed by isopods (Figure 3b); microbially processed litter was enriched in compounds N, P, and U ($P < 0.01$). On average, compound C was digested completely during isopod gut passage, and about 30% of compound E was

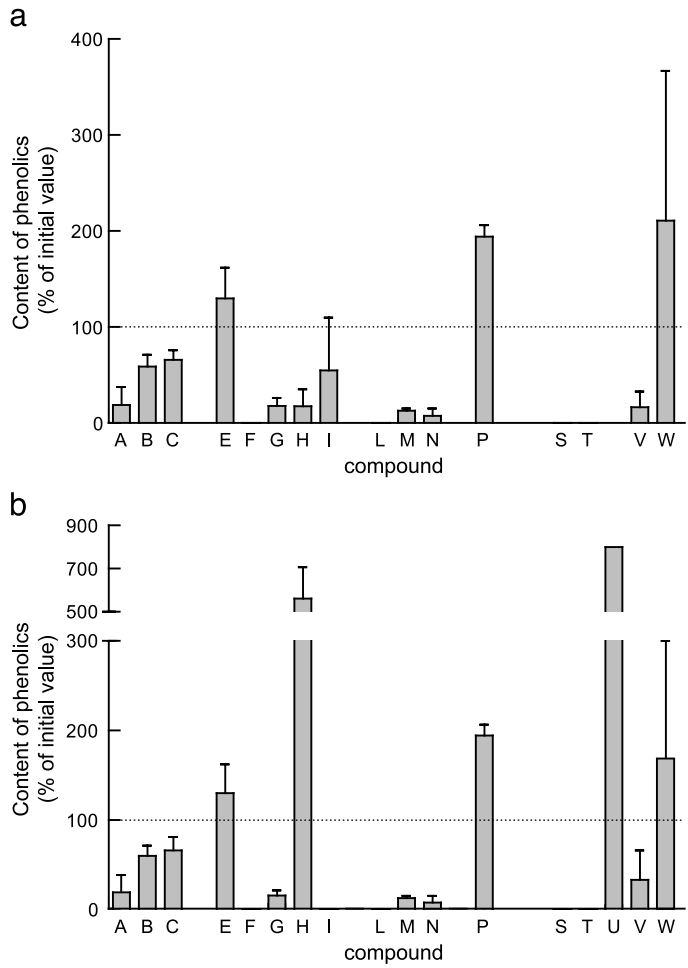

FIG. 3. Change in content of phenolic compounds in leaf litter derived from *Quercus* (a) and *Eucalyptus* (b) after 5-d incubation in aquatic environments, with *Proasellus coxalis* as detritivore, as compared with initial values of the litter (100%). Data are median ± median absolute deviation ($N = 9$). Only those peaks are listed along the *X*-axis that were found in the samples studied here.

degraded, although individual variation was high (Figure 4b), whereas compounds H, P, and U were concentrated higher in feces than in the litter ($P < 0.01$).

In the terrestrial environment, only a few compounds were completely leached, degraded, or transformed in animal-free controls (Figure 5b); only compound G was obtained in higher concentration after the experiment than

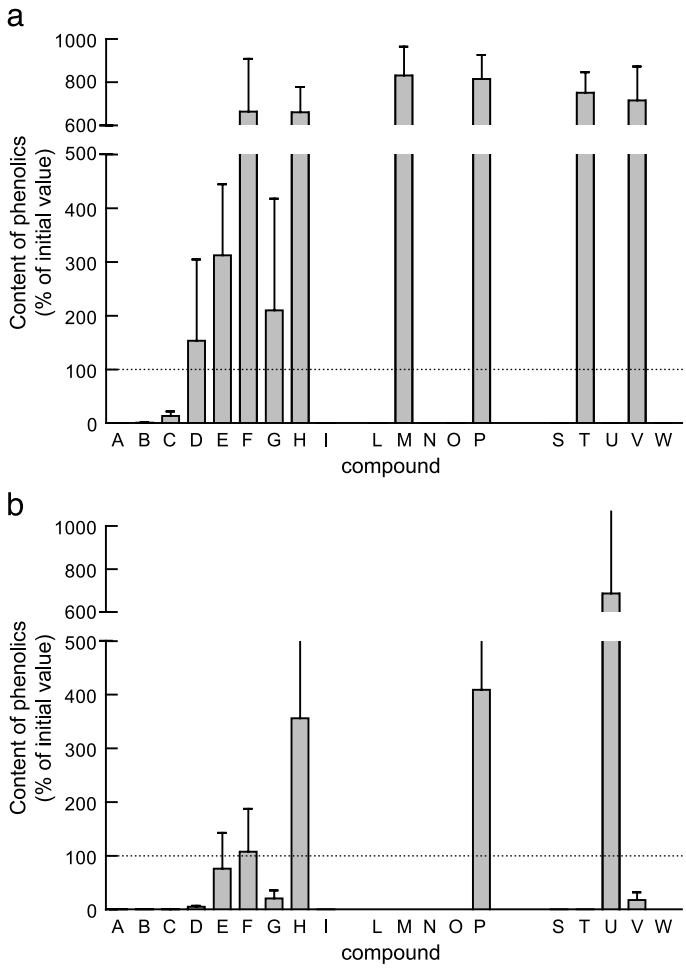

FIG. 4. Change in content of phenolic compounds of leaf litter derived from *Quercus* (a) and *Eucalyptus* (b) due to digestive processes by *P. coxalis* as deduced from comparing isopod feces with initial values of the litter (100%). Data are median ± median absolute deviation ($N = 9$). Only those peaks are listed along the *X*-axis that were found in the samples studied here.

before ($P < 0.001$). With *Porcellio* present (Figure 6b), microbially processed litter was enriched in compounds F, G, and T ($P < 0.01$). With *Eluma* present, compounds D, P, R, and V were microbially degraded or transformed in the leaf litter that was not consumed by isopods (Figure 7b), whereas compounds B, F, and T became enriched in the litter ($P < 0.01$). On average, compounds D, G, H,

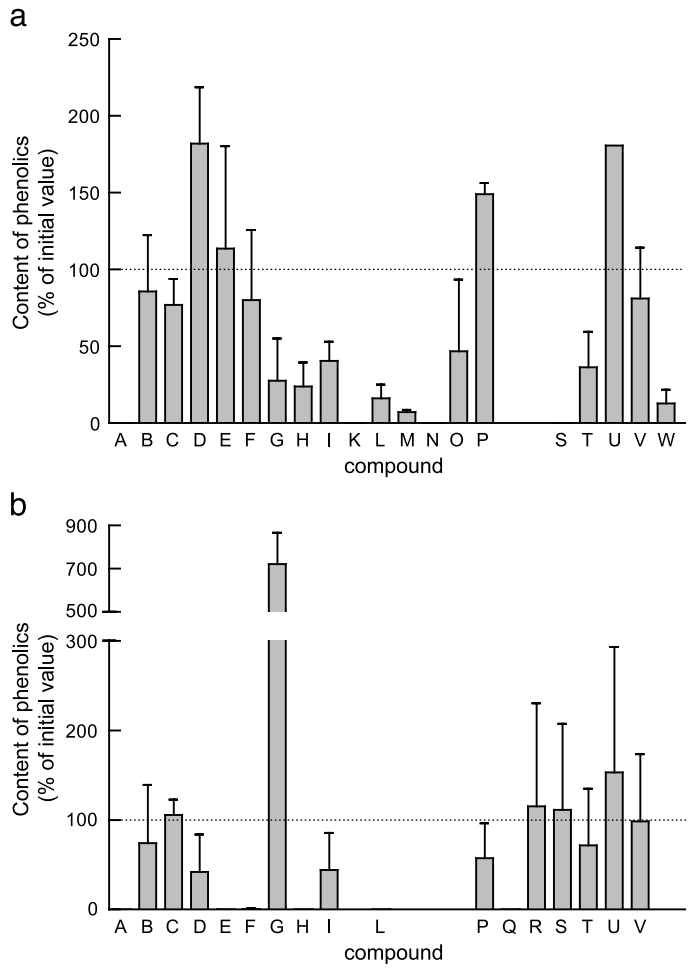

FIG. 5. Change in content of phenolic compounds in leaf litter derived from *Quercus* (a) and *Eucalyptus* (b) due to leaching and microbial processing within 5 d in terrestrial animal-free control assays as compared with initial values of the litter (100%). Data are median ± median absolute deviation ($N = 9$). Only those peaks are listed along the *X*-axis that were found in the samples studied here.

R, S, and V were digested extensively by *Porcellio* (Figure 8b), whereas compounds E, O, and T were concentrated higher in feces than in litter ($P < 0.01$). *Eluma* digested all of the remaining compounds B, G, I, P, R, T, and V, and degraded compound C almost completely (Figure 9b), but led to a relative increase of compounds D, O, and U ($P < 0.05$).

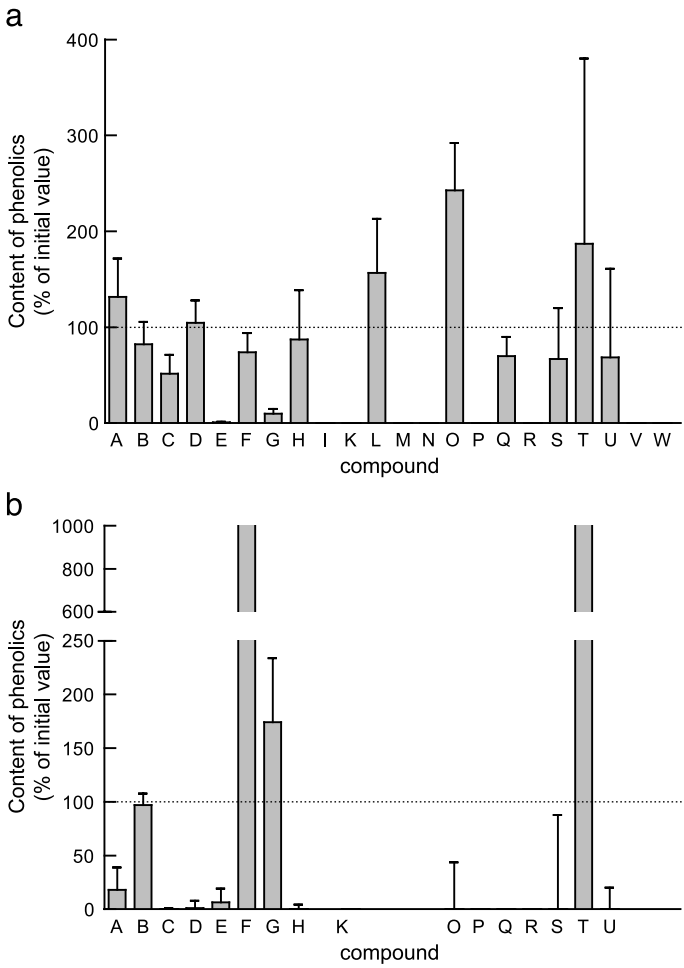

FIG. 6. Change in content of phenolic compounds in leaf litter derived from *Quercus* (a) and *Eucalyptus* (b) after 5-d incubation in terrestrial environments, with *Porcellio dispar* as detritivore, as compared with initial values of the litter (100%). Data are median ± median absolute deviation ($N = 9$). Only those peaks are listed along the *X*-axis that were found in the samples studied here.

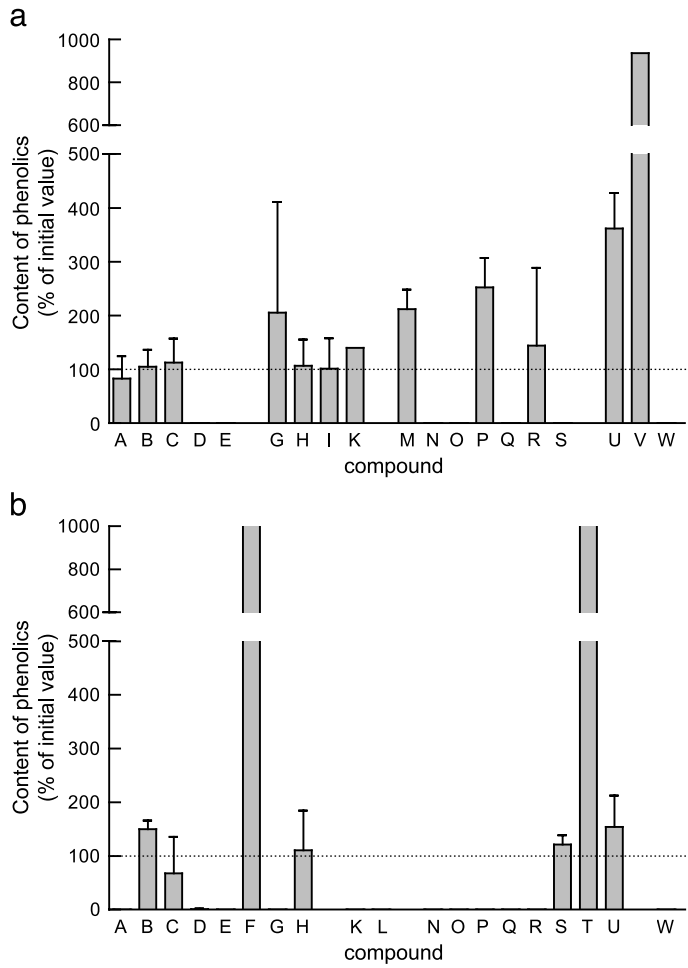

FIG. 7. Change in content of phenolic compounds in leaf litter derived from *Quercus* (a) and *Eucalyptus* (b) after 5-d incubation in terrestrial environments, with *Eluma caelatum* as detritivore, as compared with initial values of the litter (100%). Data are median ± median absolute deviation ($N = 9$). Only those peaks are listed along the *X*-axis that were found in the samples studied here.

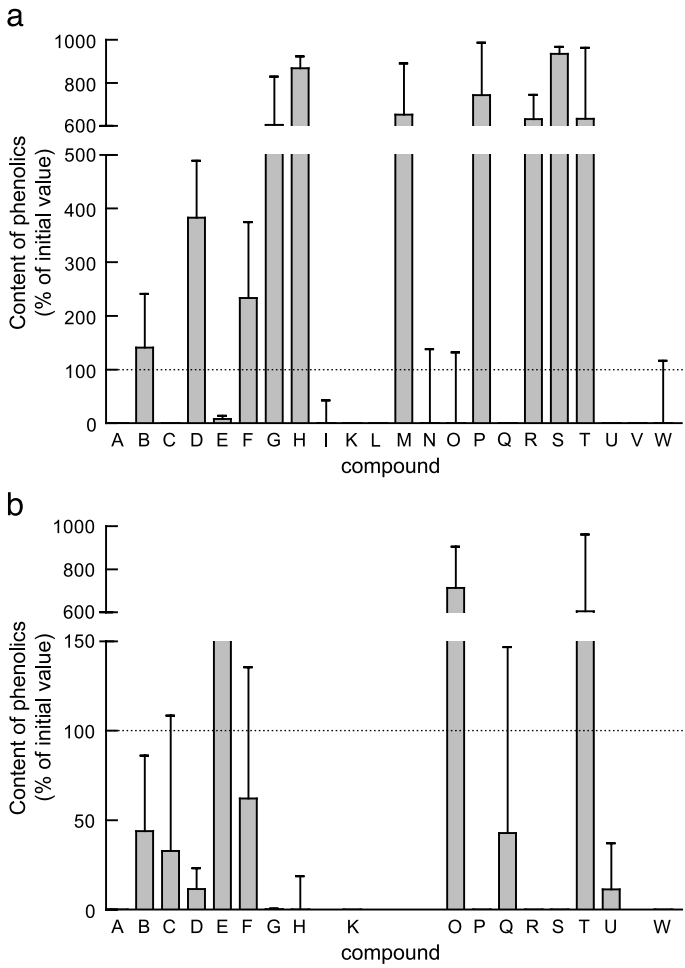

FIG. 8. Change in content of phenolic compounds of leaf litter derived from *Quercus* (a) and *Eucalyptus* (b) due to digestive processes by *P. dispar* as deduced from comparing isopod feces with initial values of the litter (100%). Data are median ± median absolute deviation ($N = 9$). Only those peaks are listed along the *X*-axis that were found in the samples studied here.

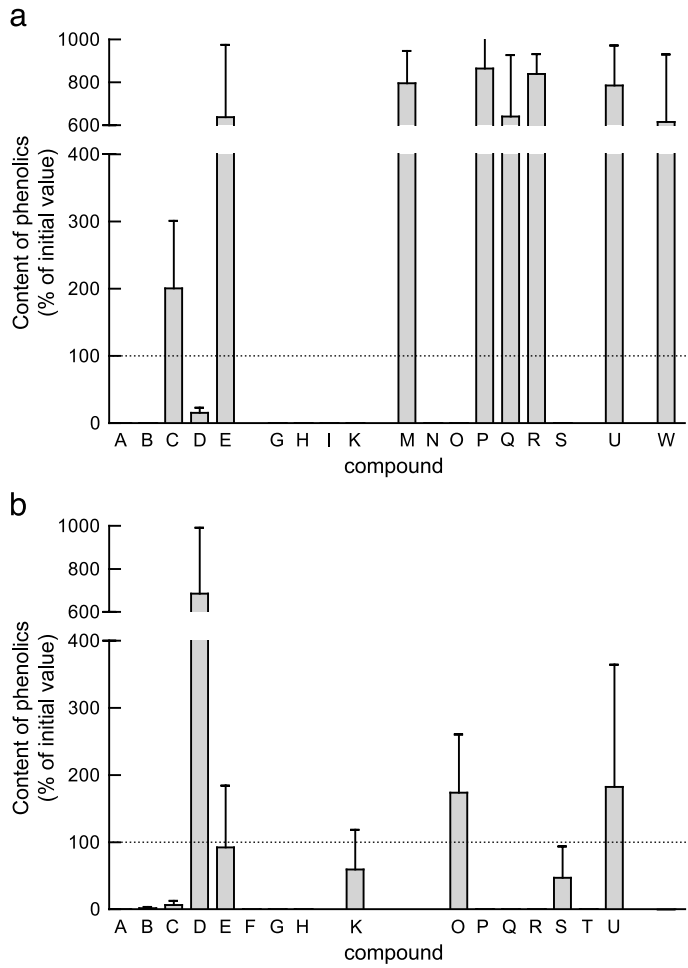

FIG. 9. Change in content of phenolic compounds of leaf litter derived from *Quercus* (a) and *Eucalyptus* (b) due to digestive processes by *E. caelatum* as deduced from comparing isopod feces with initial values of the litter (100%). Data are median ± median absolute deviation ($N = 9$). Only those peaks are listed along the *X*-axis that were found in the samples studied here.

DISCUSSION

Degradation of Particular Phenolic Compounds. Owing to the fact that we were unable to unambiguously assign different peaks of our HPLC analysis to distinct phenolic compounds (Table 1), we can only interpret our results based on rough interspecific differences between detritivores and tree species with respect to the reduction and/or increase in particular peaks. However, this information appears sufficient for a first ecosystemic approach to effects of interactions of different detritivores with different leaf litter types. Overall, however, interspecific differences deduced from our present results must be interpreted with caution because we do not know exactly whether the composition of single peaks we obtained by HPLC was the same in leaf litter from *Eucalyptus* and *Quercus*.

Ecosystemic Conclusions. The litter of *Eucalyptus* and *Quercus* we used in our study did not differ with respect to the overall content of phenolic compounds (protein-precipitating TA equivalents). The radial diffusion assay as used does not reveal phenol concentrations, but rather provides a measure of protein-binding capacity, and thus, one measure of potential biological activity of a mixture of phenolics that may or may not be correlated with phenol content. Further, tannic acid, which we used here, may not be an appropriate standard for protein precipitation by mixed phenolics because the species studied may differ in both amount and type of hydrolyzable tannins. Yet, comparison of data derived from this method still allows for an estimation of interspecific differences in phenol content or activity, and Domínguez (1994), using different methods, came to a similar conclusion of similar phenol concentrations in *Eucalyptus* and *Quercus*. Nevertheless, we found significant differences in consumption rates of these litter types by *Proasellus* but not in the terrestrial isopods. Thus, it is apparently not the total phenol content that determines detritivore consumption rates, but rather the phenolic signature.

In addition to phenolics (see Introduction), other compounds are considered responsible for mediating consumption and decay rates of plant tissue. While mainly phloroglucinol derivates, such as formylated phloroglucinol and acylphloroglucinol, are considered responsible for feeding deterrence towards mammals (e.g., Pass et al., 1998; Lawler et al., 1999; McLean et al., 2004), different essential oils of *Eucalyptus* spp. proved active against insect consumers (e.g., Lee et al., 2001; Wang et al., 2001). Whereas purely hydrocarbon components of essential oils (terpenes) are not water-soluble, and thus, will not be lost rapidly through leaching (and would have been present in the leaf litter we used in the present study if they had been so in freshly detached senescent leaves), their oxidized derivates may be leached at early decomposition stages. Thus, those essential oils that are common in *E. globulus* (e.g., 1,8-cineole and α-pinene; Li and Madden, 1995; Lee et al., 2001) are readily leached off litter (as can be deduced from, e.g., Krock et al., 2002; Rasmussen et al., 2003) and

probably were not present in our experimental litter. Those differences in consumption and digestibility of *Eucalyptus* and *Quercus* we observed herein were probably not due to essential oils. On the other hand, the lack of negative effects of *Eucalyptus* litter on decomposition through isopod feeding may be due to the lack of essential oils that had been lost from litter prior to collection.

According to our present results, phenolics in *Eucalyptus* appear to be less deterrent to the aquatic isopod, *P. coxalis*, than phenolics in *Quercus* (e.g., compounds B or I). In a previous study, *Eucalyptus* oils had stronger effects on aquatic fungal decomposers than phenolics (Canhoto et al., 2002). As expected, the loss of phenolics through leaching was almost complete and higher in the aquatic than in the terrestrial environment (but apparently had not been extensive under terrestrial conditions prior to litter collection). Thus, negative effects of *Eucalyptus* phenolics (if any exist) would be expected to be stronger for terrestrial detritivores than for aquatic, but we did not find any effect on consumption or digestibility by terrestrial isopods. From this, we conclude that decomposition of *Eucalyptus* litter does not proceed more slowly than that of litter from native Portuguese trees. In coincidence, Rezende et al. (2001) did not find significantly different decay rates of leaf litter derived from *Eucalyptus grandis*, introduced to Brazil, and *Dalbergia nigra*, a native Brazilian tree, after 1 yr.

As expected, we observed significant differences in phenol degradation, both between detritivores and between litter types. Digestive processes and effects on microbial activity obviously differ among detritivores, and both the identity of detritivores and the litter type affect phenol degradation (and probably also other decomposition processes) both directly and indirectly. Consequently, we cannot consider them functionally redundant, although they superficially belong to the same functional group (see Zimmer et al., 2002b, 2004; Chalcraft and Resetarits 2003a,b). Further, different litter types can obviously not substitute for each other in terms of their quality as food to detritivores (see Zimmer et al., 2004).

Overall, however, although the phenolic signature of *Eucalyptus* litter influences its degradation, we did not find consistent evidence for slowed-down decomposition processes of the litter of this introduced tree species that would have suggested impaired nutrient cycles. Thus, negative effects that *Eucalyptus* plantation may have on Portuguese forests and freshwater systems are not due to differences in tannins of *Eucalyptus* litter relative to common native trees.

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