Snail and millipede complementarity in decomposing Mediterranean forest leaf litter mixtures

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Summary

1. The projected increase in loss of biodiversity worldwide has prompted the need to understand the role that diversity plays in key ecosystem functions such as litter decomposition and nutrient cycling. Here we asked how two contrasting species of saprophagous macrofauna and four Mediterranean forest leaf litter species interactively affect decomposition in a 6-week microcosm study.

2. Litter mass loss and macrofauna relative consumption rates (RCR) were measured on treatments with freshly fallen or partially decomposed leaf litter of *Alnus glutinosa*, *Fraxinus angustifolia*, *Pistacia terebinthus* and *Quercus ilex* as single-species or mixtures in absence and presence of the gastropod, *Pomatias elegans*, the diplopod, *Glomeris marginata*, or both macrofauna species.

3. Macrofauna consumed all litter substrates except freshly fallen *P. terebinthus* as single-species litter that was fatal to both animals, although its presence in litter mixtures increased overall RCR. Consumption was higher in partially decomposed than freshly fallen litter, and higher in litter mixtures than in single-species litter. Both litter state and mixing interacted significantly with macrofauna treatment where generally, RCR by *P. elegans* alone was inferior to that of *G. marginata* alone or in combination with *P. elegans*.

4. An overall positive complementarity effect on litter RCR between *G. marginata* and *P. elegans* was observed in freshly fallen litter. Particularly strong complementarity was observed in two mixtures of freshly fallen litter and also in one mixture of partially decomposed litter.

5. There were no non-additive effects of litter mixing on litter mass loss in the absence of animals, indicating no interactions among litter substrates during decomposition. However, in the presence of either *G. marginata* and/or *P. elegans*, positive and negative interactions among litter substrates occurred and were enhanced or reversed by the addition of the second macrofauna species.

6. We conclude that saprophagous macrofauna play a critical role in the decomposition dynamics of Mediterranean forest litter by interacting with each other and by driving interactions among litter substrates.

Key-words: biodiversity, diplopod, gastropod, non-additive effects, synergy, litter consumption

Introduction

In recent decades, human induced activities resulting predominantly in habitat change, climate change and the introduction of invasive species have led to biodiversity losses and consequently losses in the services provided by ecosystems (Millennium Ecosystem Assessment 2005). According to recent scenarios, Mediterranean ecosystems are predicted to experience the greatest proportional change in biodiversity in coming years (Sala *et al.* 2000). With such activities projected to continue, one of the emerging priorities of biodiversity research remains to understand if and how changes in diversity and/or species composition impact ecosystem processes (Loreau, Naeem & Inchausti 2002; Hooper *et al.* 2005). Litter decomposition, a process key to nutrient cycling, has been studied in order to understand both the consequences of changes in diversity of litter types (Wardle, Bonner & Nicholson 1997;

Litter decomposers include a wide variety of different organisms ranging from microorganisms to large litter feeding soil animals such as millipedes, gastropods and earthworms (Bardgett 2005). Soil macrofauna are diverse, abundant and consume a large amount of leaf litter (Scheafer 1990; Wolters & Ekschmitt 1997; Cárcamo et al. 2000; David & Gillon 2002). They accelerate decomposition in part directly through litter consumption, but also indirectly by promoting the activity of other actors in the decomposer food web through fragmentation and transformation of litter (Theenhaus & Scheu 1996; Rawlins et al. 2006). Fauna excreta like faecal pellets, mucus and other substances provide particularly suitable substrates for microbial communities (Kautz & Topp 2000; Lavelle & Spain 2002; Wardle 2002). Nonetheless, decomposition studies often use methods that exclude macrofauna (e.g. see review by Gartner & Cardon 2004) despite recent demonstrations that their inclusion can fundamentally alter process rates, particularly when plant litter diversity (Hättenschwiler & Gasser 2005) or climate conditions are considered (Wall et al. 2008).

In Mediterranean forests, macrofaunal fresh biomass is estimated at 521 to 1131 g m⁻² (David 1999). *Glomeris marginata*, the pill millipede, can make up to 10% of this fresh biomass (58 to 108 g m⁻²) making it one of the most abundant macrodetritivores in the system (David 1999; David & Gillon 2002). Detritivorous gastropods, such as *Pomatias elegans*, can co-occur with *G. marginata*, but have a lower overall abundance (mean of *Pomatias elegans*: 47 ± 2 g live biomass m⁻²; David 1999) with a generally patchy distribution such that at smaller spatial scales, they can reach a substantial fresh biomass (David 1999). Although detritivorous gastropods receive little attention, earlier studies across Europe have underlined their importance in nutrient cycling (Frömming 1958; Mason 1970a, b; Jennings & Barkham 1979; Seifert & Schutov 1981). Saprophagous snails feed and grow well on plant litter material (Scheafer 1991a; Wolters & Ekschmitt 1997) and possess a completely different feeding strategy than earthworms, diploponds or isopods that are more commonly studied (e.g. Hassall, Turner & Rands 1987; Scheu & Schaefer 1998; Cárcamo et al. 2000; Heemsbergen et al. 2004; Zimmer, Kautz & Topp 2005).

Already some thirty years ago, Wieser (1978) recognized that different macrodetritivores (e.g. terrestrial gastropods and isopods) have strongly contrasting morphological and physiological traits that ultimately determine nutrient cycling and potentially the structure of food webs. For example, gastropods are efficient at accessing physically tough material with their radula and have the ability to synthesize major digestive enzymes making them less dependent on microbial decomposers and pre-conditioning of litter material (Newell 1967; Cameron & Redfern 1976; Wieser 1978; Scheafer 1991b). They show a very high assimilation efficiency ranging from 50 to 75% (Mason 1970b; Jennings & Barkham 1979).

In contrast, millipedes have restricted digestive capacities and lower assimilation efficiencies (Scheafer 1991b; Rawlins et al. 2006), but tend to have higher food intake rates and depend more on litter previously colonized by microorganisms (David & Gillon 2002). Under Mediterranean conditions, the assimilation efficiency of *G. marginata* is <10% when feeding on partially decomposed leaves (David & Gillon 2002). Their assimilation efficiency can triple when feeding on freshly fallen litter, but feeding trials have demonstrated a clear preference for partially decomposed litter (David & Gillon 2002). With such strongly contrasting traits, complementarity effects on decomposition seem likely, that is faster decomposition when both macrodetritivores are present. The hypothesis that species complementarity can result in enhanced ecosystem processes has received support from plant diversity-productivity experiments (Loreau & Hector 2001; Cardinalet al. 2007) and has been tested increasingly in litter decomposition systems (Jonsson & Malmqvist 2003; Heemsbergen et al. 2004; Zimmer, Kautz & Topp 2005).

Incorporating trophic complexity in biodiversity-ecosystem functioning models is challenging but recognized as important (Cardinalet al. 2006; Duffy et al. 2007). The manipulation of macrodetritivore diversity represents a single trophic level in the decomposer food web. At the bottom of the food chain, the manipulation of litter substrate diversity itself has shown that litter mixing can result in non-additive effects (slower or faster decomposition of litter mixtures than would be predicted based on single-species decomposition rates; see reviews by Gartner & Cardon 2004; Hättenschwiler, Tiunov & Scheu 2005 and recent studies by Madritch & Cardinalet al. 2007; Jonsson & Wardle 2008; Pérez Harguindeguy et al. 2008). Drivers of these non-additive litter mixing effects could include litter chemistry (Smith & Bradford 2003; Hoorens, Aerts & Strøtenera 2003; Epps et al. 2007; Liu et al. 2007) as well as decomposer organisms (Hansen & Coleman 1998; Kaneko & Salamanca 1999; Hättenschwiler & Gasser 2005).

Our study addresses the role of two contrasting species of Mediterranean forest saprophagous macrofauna and leaf litter diversity and their potential interaction in a microcosm system. More particularly, regarding macrofauna, we hypothesized that (1) freshly fallen litter (in contrast to partially decomposed litter) would be more readily consumed by the gastropod, *P. elegans*, than by the millipede, *G. marginata*; (2) the combination of *P. elegans* and *G. marginata* would result in synergistic consumption, particularly in freshly fallen litter; (3) consumption would vary with litter species composition such that (a) litter mixtures would be consumed more than single-species litter and (b) consumption of litter mixtures would increase with increasing functional dissimilarity of the mixtures. At the leaf litter diversity level, we hypothesized that (4a) non-additive litter mixing effects would be observed in the absence of macrofauna; (4b) non-additive litter mixing effects would be modified by the presence of macrofauna and (4c) non-additive litter mixing would interact with leaf litter decomposition state and litter species composition.
Materials and methods

**LEAF LITTER MATERIAL**

Four Mediterranean woody species were selected to represent a wide range of foliar traits. These were the evergreen holm oak, *Quercus ilex* L. (Q), the N-fixing deciduous black alder, *Alnus glutinosa* L. (A), the deciduous and rapidly decomposing narrow-leaved ash, *Fraxinus angustifolia* Vahl. (F), and the deciduous, slowly decomposing terebinth, *Pistacia terebinthus* L. (P). *Quercus ilex* is the dominant tree of Mediterranean forests, *A. glutinosa* and *F. angustifolia* are characteristic riparian tree species and *P. terebinthus* is a typical small tree of Mediterranean forests. These species can co-occur naturally although equal mixing is unlikely.

Freshly fallen leaf litter was collected using nets that were emptied weekly in autumn 2006 except for *Q. ilex*, which was collected in early summer 2007. While the deciduous species shed their leaves in autumn, peak litter fall of the evergreen oak is typically from late May to early July, but can extend into August depending on rainfall distribution. Collections for *F. angustifolia*, *P. terebinthus* and *Q. ilex* were in forests 30 km north-west of Montpellier, France. Collection of *A. glutinosa* was in a forest near Calmont (60 km south of Toulouse, France). Litter was oven-dried at 60 °C and only clean and intact leaves were selected. Petioles of compound leaves (*P. terebinthus* and *F. angustifolia*) were removed and leaflets were retained. Ten samples of initial litter dried at 40 °C for each of the four species was dried at 60 °C to calculate a conversion factor since the final harvest was dried at 60 °C.

In order to prepare partially decomposed litter material, freshly fallen litter was laid out in 50 × 70 cm bags of 0.5 × 1 cm mesh width and filled 2–3 cm thick. Bags were placed under *Q. ilex* forest cover in the experimental field of the CEFE-CNRS (Montpellier, France) and fixed with nails to the ground that was previously freed of naturally occurring leaf litter. Litter bags were moistened at the beginning of exposure and a second time when the bags were turned at half time. *Fraxinus angustifolia* and *A. glutinosa* were exposed for 42 days (mid December 2006 to end of January 2007). *Pistacia terebinthus* and *Q. ilex* were exposed for 65 days (mid November 2007 to mid January 2008). Following exposure, bags were oven-dried 48 h at 40 °C in order to stop microbial activity. Decomposition during the exposure period was measured independently in smaller 5 × 5 cm bags of the same thickness and mesh width and ranged from 11–12% litter mass loss for all four species.

**SOIL MACROFAUNA**

*Ponatiarias elegans* (Müller 1774) is a predominantly saprophagous terrestrial operculate snail with a body size of 0.5–1.5 cm living in calcareous zones of Western Europe and the Mediterranean. The pill millipede, *Gliomeris marginata* (Villers 1789), has a body size of 0.5–2.0 cm and is an exclusively saprophagous diplopod widespread on calcareous soils in north western and central Europe as well as in the Mediterranean. These two species co-occur widely in different types of Mediterranean forests (I.T. Handa & S. Hättenschwiler, personal observation). Animals, which are abundant and easily visible in this system, were collected manually in the litter and top soil layer of the CEFE experimental field during December 2007 (*P. elegans*) and in early February 2008 (*G. marginata*). Organisms were maintained in transparent, plastic and non-hermetic boxes (40 × 30 × 10 cm) that were filled with a substrate composed of leaf litter from where animals were collected. Litter was moistened regularly by spraying mist. Boxes were placed in a shaded experimental greenhouse that provided organisms with a natural range of temperatures (2–26 °C) and which was used thereafter for the duration of the experiment.

Prior to weighing animals, snails were cleaned gently with distilled water and dried on absorbent paper. Only snails identified as alive (emerging from their shell prior to weighing) were added to the microcosms. All millipedes were placed on a moistened paper to allow cleaning while they were moving. Among collected and cleaned organisms, weight distribution curves for each species were calculated to select organisms of similar size. *P. elegans* individuals between 350–450 mg total mass were retained (150 mg of which was shell mass on average). Correspondingly, *G. marginata* between 80–200 mg were retained. These weight ranges ensured the selection of adult organisms.

**TRAIT MEASUREMENT AND FUNCTIONAL DIVERSITY OF LEAF LITTER MIXTURES**

Four functional traits were selected in order to calculate functional diversity of the leaf litter mixture treatments (Table 1). These traits, which can affect litter decomposition, were nitrogen and lignin concentrations, foliar resistance and tri-dimensionality (spatial conformation) (Hättenschwiler, Tianou & Scheu 2005). To prepare litter for chemical analyses, three replicates of each litter type were ground separately with a 1 mm screen on a Cyclotec 1093 Sample Mill (Foss Tectator, Höganas, Sweden). Nitrogen concentration was measured with a Thermo-Finnigan NC EA 1112 elemental analyser (Strada Rivolta, Milan, Italy). Lignin concentration was determined by the classical fibre analysis (van Soest 1963). Foliar resistance was measured with a penetrometer device where the weight of the amount of water needed to pierce a clamped wet leaf with a 1.55 mm needle was measured (Grac¸a & Zimmer 2004). Tri-dimensionality was estimated by counting the number of leaves fitted into a given volume without packing or arranging leaves.

For each litter species, the mean trait value obtained within one species was standardized by subtracting the mean across all species and dividing by the range across all species for a particular trait (Botta-Dukát 2005). After standardization, the four traits were used to assess dissimilarity for all two- and three-species litter mixtures. Functional dissimilarity was calculated according to the Rao/quadricatic entropy method where

\[
FD_{0} = \sum_{i} \sum_{j} d_{ij} p_{ij}
\]

and *i* and *j* are the two leaf litters species, *d* is the Euclidean distance balanced by the number of traits and *p* the relative abundance (Botta-Dukát 2005). Calculations were done with the software R (version 2.4.0; R Development Core Package 2006) using the package ‘diversity’. From these values of functional dissimilarity, six mixtures of leaf litters were selected to obtain one similar, intermediate and dissimilar combination for both two- and three-species mixtures. Two-species mixtures were *A. glutinosa* – *F. angustifolia* (AF), *F. angustifolia* – *Q. ilex* (FQ) and *A. glutinosa* – *Q. ilex* (AQ). Three-species mixtures were *A. glutinosa* – *F. angustifolia* – *P. terebinthus* (AFP), *F. angustifolia* – *Q. ilex* – *P. terebinthus* (FQP) and *A. glutinosa* – *Q. ilex* – *P. terebinthus* (AQP). In increasing order of dissimilarity, the gradient ranged from the mixture AF (functional dissimilarity coefficient value: *fd* = 0.20), AQP (*fd* = 0.28), FQP (*fd* = 0.36), FQ (*fd* = 0.41), AQ (fd = 0.69) and AQ (fd = 0.90). In order to test each trait for differences among species and litter decomposition state, data were log-transformed and analysis of variance with TukeyHSD tests were used.
Our experimental design consisted of ten levels of leaf litter species composition (four single species combinations, and three each of two- and three-species mixtures as described above). These were crossed with four levels of macrofauna treatments: absence of animals, P. elegans only, G. marginata only and a combined treatment of P. elegans and G. marginata. In addition, all litter × macrofauna treatments were crossed with either freshly fallen or partially decomposed leaf litter. All combinations were replicated four times yielding a total of 320 microcosms.

We used transparent, non-hermetically closed, rigid plastic boxes (115 × 85 × 43 mm) (LAB no. 3; Caubères, Yebles, France) as microcosms. A total of 2.6 g of leaf litter was added to each microcosm representing a non-limiting quantity of food for the macrofauna. Leaf litter species in mixtures were of an equal proportion. Microcosm assembly began on 11 February 2008 and was done in three batches which all were completed by 15 February 2008. Leaf litter was moistened with 2 mL of distilled water mist immediately after adding litter to the microcosms, and an additional 4 mL, 24 h later. After 48 h of equilibration, a total of two individuals of macrofauna were added to each microcosm according to the specific treatments (corresponding to the microcosm to a range of biomass between 400 and 600 mg for P. elegans (with shell correction), 160 and 400 mg for G. marginata and 280 to 500 mg when both organisms were present. Litter humidity was kept constant by regularly spraying mist (usually 1 to 3 mL every 3 days).

Microcosms were kept in a non-regulated shaded greenhouse allowing for natural temperature variation. Each batch was adjacent to each other in three juxtaposed zones. Microcosms were randomized within batches and each batch was shifted three times ensuring that all batches were in all zones for an identical length of time. We checked weekly for dead animals, which were replaced with individuals of a similar weight at any first mortality event (<10%), but not thereafter. Microcosms with dead animals after a first mortality replacement occurred only in the freshly fallen single species P. terebinthus treatment, which were subsequently excluded from all analyses.

Microcosms were harvested from 25–28 March 2008 in the same manner. A total of 6 g of leaf litter was added to each microcosm representing a non-limiting quantity of food for the macrofauna. Leaf litter mass loss data permitted us to contrast the litter species composition in microcosms with dead animals after a first mortality replacement with that of microcosms with no animals added to them to those with an animal treatment in order to understand the impact of soil macrofauna on interactions among leaf litters. Although we recognize that animal biomass was not constant in all microcosms (but held comparatively similar by choosing individuals from a large pool of animals), this extra noise was present across all leaf litter mixtures comparatively similar by choosing individuals from a large pool of animals, this extra noise was present across all leaf litter mixtures. The use of RCR allowed us to compare consumption across all animal treatments since total animal biomass was not constant in all microcosms. Analysis of variance was used to test for the effect of species and litter decomposition state on the four measured leaf litter traits. To test hypotheses (1) and (3a) related to relative consumption rate, a full analysis of variance model with sequential sums of squares was used to fit leaf litter decomposition rate, macrofauna treatment and leaf litter species composition. The variation due to litter species composition was decomposed into a contrast for litter mixing (single-species vs. litter mixtures) and a residual species composition contrast. All post hoc contrasts were done using Tukey HSD. A linear regression model was used to test for a relation between RCR and functional dissimilarity (hypothesis 3b). In order to evaluate whether fauna species combination or litter mixtures resulted in non-additive interactions (synergy or antagonism) or neutral additive effects on RCR, predicted values were calculated by taking the mean of single species litter treatments (with the corresponding macrofauna treatment). Standard errors for predicted values were calculated across one given mixture treatment for replicates obtained by random combination of single-species treatments. Potential synergy of macrofauna species (hypothesis 2) was tested by contrasting predicted and observed RCR.

The leaf litter mass loss data permitted us to contrast the microcosms with no animals added to them to those with an animal treatment in order to understand the impact of soil macrofauna on interactions among leaf litters. Although we recognize that animal biomass was not constant in all microcosms (but held comparatively similar by choosing individuals from a large pool of animals), this extra noise was present across all leaf litter mixtures and any significant effects on non-additive litter species interactions that appear otherwise can be interpreted as being particularly strong. We calculated deviance (difference of observed and predicted values relative to predicted values; sensu Loreau 1998) as a response variable so as to test the influence of leaf litter state, macrofauna treatment and species litter composition on non-additive interactions (hypotheses 4 and 5). All statistical analyses were performed using R (version 2.4.0; R Development Core Team 2006).
Results

LEAF LITTER CHARACTERIZATION

Litter traits varied strongly among species (Table 1, \( P < 0.0001 \) for each trait tested). In freshly fallen leaf litter, \( A. \ glutinosa \) had the highest N concentration and tri-dimensionality (lowest index values). The highest lignin concentration and foliar resistance was found in \( Q. \ ilex \). Species ranking was largely conserved in partially decomposed leaf litter with the exception of lignin concentration, where \( P. \ terebinthus \) had as high concentrations as \( Q. \ ilex \) and for spatial conformation, where \( F. \ angustifolia \) lost tri-dimensionality when partially decomposed (Table 1). Across species, there were significant differences between partially decomposed and freshly fallen litter for all litter traits (\( P < 0.05 \)). The most pronounced difference was for lignin concentration, which was 18 to 82% higher in partially decomposed litter (Table 1).

RELATIVE CONSUMPTION RATE BY MACROFAUNA

Litter RCR by macrofauna was influenced significantly by all our main factors, namely litter decomposition state, macrofauna treatment, species composition of litter and single-species vs. litter mixtures (Table 2). Both litter decomposition states were consumed by macrofauna ranging from 3 to 27 mg \( g^{-1} \) day\(^{-1} \) in freshly fallen and 2 to 41 mg \( g^{-1} \) day\(^{-1} \) in partially decomposed litter. Relative consumption rate was 65% higher in partially decomposed than freshly fallen leaf litter (Fig. 1; Table 2; \( P < 0.001 \)). Overall, multi-species litter mixtures (16 ± 3 mg \( g^{-1} \) day\(^{-1} \)) were consumed 22% more than single-species litter (13 ± 2 mg \( g^{-1} \) day\(^{-1} \)). Table 2; \( P < 0.005 \), and there were strong differences among litter species composition treatments (Fig. 1).

Independently of litter state or litter composition, \( P. \ elegans \) or \( G. \ marginata \) alone on average consumed 10 ± 1 mg \( g^{-1} \) day\(^{-1} \) and 20 ± 3 mg \( g^{-1} \) day\(^{-1} \) respectively. In combination, both animals consumed 17 ± 2 mg \( g^{-1} \) day\(^{-1} \). The effect of the macrofauna treatment interacted significantly with litter state and in a three-way interaction with litter state and litter mixing (Table 2). To interpret these interactions, reduced statistical models and post hoc comparisons revealed the following. The strong interaction between macrofauna and decomposition state was driven by the more than double RCR of \( G. \ marginata \) alone in partially decomposed compared to freshly fallen litter (Fig. 1; Table 2; \( P < 0.001 \)).

\[ \text{Table 2. Analysis of variance testing for the effect of leaf litter state, macrofauna treatment, litter mixing (single species vs. mixtures) and litter composition on relative consumption rate by macrofauna} \]

<table>
<thead>
<tr>
<th></th>
<th>d.f.</th>
<th>SS</th>
<th>MSS</th>
<th>F</th>
<th>P</th>
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<td>1036</td>
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<tr>
<td>Litter mixing (LM)</td>
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<td>639</td>
<td>8.5</td>
<td>&lt;0.005</td>
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<tr>
<td>Composition (C)</td>
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<td>502</td>
<td>6.8</td>
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Fig. 1. Relative consumption rate of leaf litter shown by macrofauna treatment for single species and mixtures on (a) freshly fallen and (b) partially decomposed leaf litter (mean ± SE, \( n = 4 \)). Mixtures are represented from left to right with increasing functional dissimilarity. \( Pistacia \ terebinthus \) values of freshly fallen litter are not available (NA) due to macrofauna mortality in this treatment.

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consumed more partially decomposed in contrast to freshly fallen litter (+31% and +46%) but was much less discriminating towards litter states (Fig. 1; Table 2; \( P < 0.032 \)). When litter mixing was added to the macrofauna by litter state interaction, RCR by \( P. \) elegans alone was always inferior to that of \( G. \) marginata alone for both litter states in single-species litter. There was no clear emerging pattern when both detritivores were combined on single-species litter. In litter mixtures, RCR by \( P. \) elegans alone was in all cases inferior to treatments with \( G. \) marginata (either alone or in combination with \( P. \) elegans) with four exceptions. These were \( G. \) marginata alone in freshly fallen mixtures of AF and FQ, and both organisms together in freshly fallen and partially decomposed mixtures of FQ.

The importance of litter species composition was evident as the best consumed single-species litters across macrofauna treatment or litter state were \( A. \) glutinosa (3 to 40 mg g\(^{-1}\) day\(^{-1}\)) and \( Q. \) ilex (8 to 29 mg g\(^{-1}\) day\(^{-1}\); Fig. 1). \( F. \) angustifolia as a single-species litter was also well consumed (2 to 21 mg g\(^{-1}\) day\(^{-1}\)). \( P. \) terebinthus was the least consumed single-species litters with a range of 0 to 14 mg g\(^{-1}\) day\(^{-1}\) (Table 2; \( P < 0.005 \)). In fact, as freshly fallen litter, consumption of \( P. \) terebinthus resulted in 83% mortality of \( P. \) elegans and 53% mortality of \( G. \) marginata during the first thirty days of the experiment. However, the addition of \( P. \) terebinthus in litter mixtures increased overall consumption (Fig. 1).

Relative consumption rate of litter mixtures was not correlated to their functional dissimilarity (\( R^2 = 0.001, \ P = 0.645 \)). However, \( G. \) marginata alone tended to consume more in the intermediate and highly dissimilar mixtures in partially decomposed litter (37 ± 2 mg g\(^{-1}\) day\(^{-1}\) on average for the mixtures FQP, FQ, AQP and AQ; Fig. 1). No trend was observed for \( P. \) elegans alone or in combination with \( G. \) marginata.

**SYNERGY BETWEEN POMATIAS ELEGANS AND GLomeris Marginata**

In order to address the hypothesis of macrofauna complementarity, predicted and observed RCR of the combined \( P. \) elegans and \( G. \) marginata treatment (based on their respective single animal species treatments) were contrasted. These tests were done for freshly fallen and partially decomposed litter separately since litter state explained the highest amount of variance in an overall analysis (\( F = 31.6, \ P < 0.0001 \)). In freshly fallen litter, there was an overall significant difference between observed and predicted values supporting the synergy hypothesis (Fig. 2; \( F = 6.4, \ P = 0.001 \)). As there was also a strongly significant litter species composition effect (\( F = 6.8, \ P < 0.0001 \)), tests were done for each species composition separately (Fig. 2). These revealed only two clearly significant positive non-additive cases (AQP and AQ, \( P < 0.01 \); Fig. 2), despite the overall effect. These effects represented a +71% and +61% increase in RCR in presence of both the snail and millipede for AQP and AQ respectively. In contrast, in partially decomposed litter, there was no overall significant synergy of the animals observed, although litter species composition was strongly significant (\( F = 4.4, \ P < 0.001 \)), and there was a significant interaction between the predicted vs. observed values and litter species composition (\( F = 2.5, \ P < 0.05 \)). When analysed separately by litter treatment, only the FQ mixture showed a positive, non-additive significant effect (\( F = 8.42, \ P < 0.05 \); data not shown).

**NON-ADDITIVE LITTER MIXTURE EFFECTS ON MASS LOSS**

The overall test for non-additivity indicated that mean deviance values lay within a confidence interval > 0 (\( \mu = 0.12 \) to 1.05; \( P = 0.014 \), and thus showed a preponderance towards positive non-additive effects. The influence of our treatments on deviance indicated that this non-additivity was driven by the interaction between decomposition state and macrofauna treatment (\( P < 0.001 \); Table 3). In freshly fallen leaf litter, there were no significant non-additive effects on leaf mixtures in the presence of \( P. \) elegans only, except for the mixture FQP which showed a +12% positive effect (Fig. 3a; \( P < 0.05 \)). When \( G. \) marginata was added to \( P. \) elegans, this positive effect (+19%) remained and three additional treatments showed also a positive significant effect with +13–18% difference between predicted and observed mass loss (Fig. 3a; \( P < 0.05 \)). These effects were not observed if \( G. \) marginata was alone, suggesting that non-additive effects on litter mass loss were due to the combined activity of both animal species. In contrast, in partially decomposed litter, patterns were entirely different. In the presence of \( G. \) marginata only, the mixtures of FQP and FQ showed a positive non-additive effect of +33% and +21% respectively (Fig. 3b; \( P < 0.05 \)). In these cases, the addition of \( P. \) elegans to \( G. \) marginata resulted in no difference between observed and predicted.
values. No effects were observed in the presence of *P. elegans* alone.

There were no significant non-additive effects on litter mass loss in the absence of animals (data not shown). The overall significant non-additive effects of litter mixing on litter mass loss was thus entirely driven by the presence of macrofauna in the microcosms. For reference, litter mass loss in animal free treatments due to microbial activity and leaching was on average 11 ± 1% in freshly fallen litter and 7 ± 1% in partially decomposed litter (data not shown). Litter mass loss in macrofauna presence (across all animal treatments) was on average 14 ± 2% in freshly fallen litter and 13 ± 2% in partially decomposed litter.

### Discussion

Our results clearly demonstrate that litter detritivores play a fundamental role in the process of litter decomposition and how it is influenced by mixing of litter species. In our experiment which allowed for a direct contrast with animal free treatments, macrofauna contributed up to 43% of total litter mass loss across all treatment combinations. These measurements might underestimate the macrofauna component in the natural situation, because total macrofauna biomass in Mediterranean forests is typically up to 2 or 3-fold higher than it was in our experiment where an average fresh animal biomass of 40 g m⁻² was used (David 1999). Relative consumption rate in our experimental system varied from 10–20 mg g⁻¹ fresh macrofauna day⁻¹. These compared well to rates of 11–62 mg g⁻¹ day⁻¹ reported for *G. marginata* feeding on slightly decomposed *Quercus ilex* leaves (<3 months) and in the absence of soil by David & Gillon (2002) in their lab feeding study under seasonally variable Mediterranean conditions. Litter RCR by *P. elegans* have not been measured to our knowledge, however RCR of woodland snails in lab litter feeding trials of temperate deciduous species range from 3–16 mg litter g⁻¹ day⁻¹ (Mason 1970b; Seifert & Schotz 1981). Relative consumption rates of slugs have been measured feeding on evergreen *Quercus* litter in India at 40 ± 0.6 mg litter g⁻¹ day⁻¹ (Gupta & Oli 1998) and on deciduous *Sambucus, Fraxinus* and *Quercus* litter in Britain between 7.7 and 16.5 mg litter g⁻¹ day⁻¹ (Jennings & Barkham 1979).

The four selected litter species were consumed in both decomposition states to varying degrees with one important exception: neither *G. marginata* nor *P. elegans* were able to feed on freshly fallen *P. terebinthus* litter and suffered from high mortality rates when exposed exclusively to this litter. *Pistacia terebinthus* is known to be rich in plant secondary compounds, such as essential oils, sterols, tannins, tocopherols and fatty acids (Matthaus & Ozcan 2006; Coulis et al. 2009). It is likely that these compounds are still present and are toxic in fresh litter at sufficiently high concentrations to render it toxic to litter-feeding detritivores as observed in *Fagus* on earthworms, millipedes and woodlice (*Cárpeno* et al. 2000; Hedde et al. 2007). However, in partially decomposed *P. terebinthus* litter, these toxic compounds may have been sufficiently leached or degraded by microbes resulting in RCR for both animal species that were comparable to other litter treatments in our study and no abnormal mortality. Hence, for particular litter species containing toxic compounds, feeding strategies might be similar in otherwise functionally different snails and millipedes. Interestingly, the combined presence of freshly fallen *P. terebinthus* with other litter species in mixtures not only cancelled out the strong negative *P. terebinthus* effect on animals, but actually increased overall RCR. While this result is a very clear example of how litter mixing can change the effects of single litter species on decomposers, we can only speculate that the toxic compounds have either been chemically neutralized by compounds released from the other.

litter species or that the animals increased the consumption of the accompanying litter species in order to better tolerate the toxic compounds from *P. terebinthus*.

**SOIL FAUNA INTERACTIONS**

Interactions among co-occurring saprophagous macrofauna have been rarely studied with, to our knowledge, only a few exceptions (Heemsbergen et al. 2004; Hättenschwiler & Gasser 2005; Zimmer, Kautz & Topp 2005). Our first hypothesis, predicting that *P. elegans* will more readily consume freshly fallen litter in contrast to *G. marginata*, was to test a potential mechanism that could result in synergistic RCR of both litter-feeding organisms. This hypothesis was validated with our results showing that RCR of *P. elegans* did not vary between litter states. Although, both species fed on freshly fallen leaf litter, *G. marginata* showed 100% higher RCR of partially decomposed litter than *P. elegans*, confirming its clear preference for the latter as previously shown by David & Gillon (2002) using *Q. ilex* litter. This distinct feeding behaviour between *G. marginata* and *P. elegans* underlines the functional difference between millipedes and snails.

Based on the expected functional differences between the snail and millipede, our second hypothesis predicted that the simultaneous presence of *G. marginata* and *P. elegans* would result in a complementarity effect, that is higher litter RCR together than would be expected from each animal species separately. The comparison between observed RCR in microcosms with both animal species present and the predicted RCR from microcosms with either *G. marginata* or *P. elegans* present showed in three cases a significant positive effect of interacting animals on RCR of either freshly fallen or partially decomposed litter (Fig. 2). However, observed synergy was stronger in freshly fallen litter and depended on species composition of litter. Synergistic interactions among different species of litter feeding animals have also been observed for three Plecoptera (insects) species in an aquatic ecosystem (Jonsson & Malmqvist 2000), for eight different soil macrofauna species (Heemsbergen et al. 2004), and for the combination of an earthworm and an isopod species (Zimmer, Kautz & Topp 2005). All these studies were done in controlled laboratory conditions like our study, and used *Alnus* spp. as the single litter species, except for Zimmer, Kautz & Topp (2005) who used *Quercus robur* litter in addition to *Alnus glutinosa*. The only published field study manipulating macrofauna presence we are aware of, found no synergistic effects between *Glomeris* spp. and an anecic earthworm species exposed to a range of different litter species and mixtures (Hättenschwiler & Gasser 2005).

There are basically two potential mechanisms that might explain synergistic effects between *G. marginata* and *P. elegans* on litter RCR: niche differentiation (resource partitioning) and facilitation. These mechanisms are widely discussed in the literature of plant diversity effects on net primary production and are thought to contribute to complementarity in more diverse plant communities (Loreau & Hector 2001; Cardinale et al. 2007). It is notoriously difficult to separate these mechanisms (van Ruijven & Berendse 2003; Roscher et al. 2008), and it is likely that both contributed to the observed increased RCR. The more pronounced synergistic effects in freshly fallen litter may suggest facilitation by *P. elegans* which fed well on freshly fallen and partially decomposed litter. In the former case, *P. elegans* may facilitate access for the litter-chewing *G. marginata* by scraping off the tough cuticle. Additionally, *P. elegans* could accelerate microbial colonization of litter by exposing inner cell layers through feeding, by the production of wet faecal pellets (Nelson 1976; Gupta & Oli 1998) and by mucus secretion (Theenhaus & Scheu 1996). In turn, well-colonized litter fragments would be then increasingly consumed by *G. marginata*. Facilitation has been suggested as the main mechanism for increasing litter mass loss along a gradient of increasing functional dissimilarity of macrofauna communities (Heemsbergen et al. 2004). Although Heemsbergen et al. (2004) did not include gastropods, it is likely that the combination of *P. elegans* and *G. marginata* with their contrasting physiological and behavioural traits would be at the higher end of their functional dissimilarity gradient. Zimmer, Kautz & Topp (2005) also interpreted observed synergy between an isopod and an earthworm species feeding on alder litter as facilitation, although they found no synergistic effects when both animals fed on single-species oak or on an alder-oak mixture. In our study, complementarity of *G. marginata* and *P. elegans* on litter RCR depended clearly on species composition of litter, which may help interpret the underlying mechanisms.

Facilitation among the macrofauna species alone does not seem to explain the observed results, because one would expect similar synergistic interactions in both single-species treatments and litter mixtures. The fact that the strongest synergistic interactions were observed in litter mixtures, suggests that resource partitioning is also at play. More complex food sources may be exploited more efficiently by functionally diverse animal communities, providing some evidence for niche differentiation among litter detritivores. It is interesting to note that although functional dissimilarity (which we calculated based on chemical and structural traits) did not explain overall RCR patterns, the two mixtures where clear evidence of synergy occurred among animals (*Alnus-Quercus* and *Alnus-Quercus-Pistacia*) were those with the highest functional dissimilarity of all six mixtures (Fig. 2). In conclusion, litter type and detritivore community composition interactively determine rates of litter consumption in a complex way. Complexity arises because different mechanisms, such as facilitation and resource partitioning, appear to be involved in animal interactions, and the relative importance of these mechanisms seems to change dynamically depending on litter species composition and the decomposition state of the litter.

**LITTER MIXING EFFECTS ON MASS LOSS**

While macrofauna RCR contributed 21–40% of the total litter mass lost in this system, all remaining mass loss was attributed to leaching and microbial activity. In the absence of animal activity, observed mass loss in the six litter mixtures...
was not significantly different than predicted mass loss of mixtures based on single species litter treatments, thus rejecting our hypothesis that such differences would occur. This result contrasts with recent reviews showing that in the majority of the published studies at that time, litter mixing had non-additive effects on litter decomposition (Gartner & Cardon 2004; Hättenschwiler, Tiunov & Scheu 2005). However, as pointed out by Hättenschwiler, Tiunov & Scheu (2005), comparisons among different studies are problematic because the dynamic process of litter decomposition has been interrupted at different stages, and mixing effects might vary through time (McTiernan, Ineson & Coward 1997). Our short-term study covered only the initial phase of litter decomposition, with an important contribution of litter-species specific leaching processes to the overall mass loss. The combined leaching and microbial degradation accounted for 2.6 ± 0.6% and 17.9 ± 1.4% mass loss in the most slowly (Quercus ilex partially decomposed) and the most rapidly (Fraxinus angustifolia freshly fallen) decomposing litter treatment respectively. In decomposition experiments over longer time periods and under field conditions, microbial driven non-additive litter mixture effects have frequently (Madritch & Cardinale 2007; Pérez Harguindeguy et al. 2008) but not always (Ball et al. 2008; Vivanco & Austin 2008) been observed, and can depend on environmental context (Madritch & Cardinale 2007; Jonsen & Wardle 2008).

All the significant mixture effects observed on litter mass loss were fauna driven. In freshly fallen leaf litter mixtures, non-additive effects were observed marginally when P. elegans was present, but reinforced significantly by the addition of G. marginata (Fig. 3a), underlining the key role of P. elegans for fresh litter decomposition. In contrast, G. marginata was driving the significant mixture effects observed in partially decomposed litter, and the addition of P. elegans neutralized these effects (Fig. 3b). These observations generally indicate that the presence of litter feeding macrofauna and their identity is key for understanding litter mixture effects on decomposition. Our study equally emphasizes, that the exclusion of soil invertebrates can have important impacts on estimations of process rates in line with previous studies (Hättenschwiler & Gasser 2005; Schäder & Brandl 2005; Zimmer, Kautz & Topp 2005). In a recent global decomposition experiment, Wall et al. (2008) have argued that the impact of soil fauna is strongest in temperate and wet tropical climates, but neutral where temperature or moisture constrains biological activity. Although there is strong seasonal variation in feeding activity of macrofauna in the drier Mediterranean climate (David & Gillon 2002), our data suggest that during their active periods, their impact is equally important but dependent on litter decomposition state, litter species composition and fauna community composition. In conclusion, litter feeding macrofauna in interaction with litter composition may determine organic matter turnover and nutrient cycling in Mediterranean forests in important ways, requiring their explicit consideration for the assessment of the effects of changing biodiversity and global change for the functioning of Mediterranean forest ecosystems.

Acknowledgements

We thank JF David for his advice, encouragement and literature on soil macrofauna; S Barantal and S Coq for discussions on calculating functional similarity and statistics; B Buatois, R Leclerc and E Chauvet for advice on trait measurement; C Collin, D Delguedre and C Escape for technical help; JM Ourcival for help during litter collection; JI Salager, M Coulis and L Sonié for sorting litter and our colleagues at the CEFE and in the BioCycle consortium for their general support and discussions. We gratefully acknowledge the careful comments of the anonymous referees. This study is an individual subproject contributing to the BioCycle collaborative research project funded by the EUROCORES programme EuroDIVERSITY of the European Science Foundation (ESF). BioCycle is endorsed by DIVERSITAS as contributing towards their current scientific research priorities in biodiversity science. We dedicate this article to Jean De Oliveira who was a supportive admirer of research, education and papyrus-litter-feeding macrofauna.

References


